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(57) Abstract

Disclosed herein are non–endogenous, constitutively activated forms of the human 5–HT_{2A} and human 5–HT_{2C} receptors and uses of such receptors to screen candidate compounds. Further disclosed herein are candidate compounds identified by the screening method which act at the $5HT_{2A}$ receptors. Yet further disclosed is a new class of compounds which act at the $5HT_{2A}$ receptors.

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NON-ENDOGENOUS, CONSTITUTIVELY ACTIVATED HUMAN SEROTONIN RECEPTORS AND SMALL MOLECULE MODUALTORS THEREOF

The benefit of U.S. Serial Number 09/060,188, filed April 14, 1998 (owned by Arena Pharmaceuticals, Inc.) and U.S. Provisional Number 60/090,783, filed June 26, 1998 (owned by Arena Pharmaceuticals), U.S. Provisional Number 60/112,909, filed December 18, 1998, and U.S. Provisional Number 60/123,000 filed March 5, 1999 is hereby claimed.

FIELD OF THE INVENTION

The present invention relates to non-endogenous, constitutively active serotonin receptors and small molecule modulators thereof.

BACKGROUND OF THE INVENTION

I. G protein-coupled receptors

G protein-coupled receptors share a common structural motif. All these receptors have seven sequences of between 22 to 24 hydrophobic amino acids that form seven alpha helices, each of which spans the membrane. The transmembrane helices are joined by strands of amino acids having a larger loop between the fourth and fifth transmembrane helix on the extracellular side of the membrane. Another larger loop, composed primarily of hydrophilic amino acids, joins transmembrane helices five and six on the intracellular side of the membrane. The carboxy terminus of the receptor lies intracellularly with the amino terminus in the extracellular space. It is thought that the loop joining helices five and six, as well as, the carboxy terminus, interact with the G protein. Currently, Gq, Gs, Gi, and Go are G proteins that have been identified. The general structure of G protein-coupled receptors is shown in Figure 1.

Under physiological conditions, G protein-coupled receptors exist in the cell membrane in equilibrium between two different states or conformations: an "inactive" state and an "active" state. As shown schematically in Figure 2, a receptor in an inactive state is unable to link to the intracellular transduction pathway to produce a biological response. Changing the receptor conformation to the active state allows linkage to the transduction pathway and produces a biological response.

A receptor may be stabilized in an active state by an endogenous ligand or an exogenous agonist ligand. Recent discoveries such as, including but not exclusively limited to, modifications to the amino acid sequence of the receptor provide means other than ligands to stabilize the active state conformation. These means effectively stabilize the receptor in an active state by simulating the effect of a ligand binding to the receptor. Stabilization by such ligand-independent means is termed "constitutive receptor activation."

II. Serotonin receptors

Receptors for serotonin (5-hydroxytryptamine, 5-HT) are an important class of G protein-coupled receptors. Serotonin is thought to play a role in processes related to learning and memory, sleep, thermoregulation, mood, motor activity, pain, sexual and aggressive behaviors, appetite, neurodegenerative regulation, and biological rhythms. Not surprisingly, serotonin is linked to pathophysiological conditions such as anxiety, depression, obsessive-compulsive disorders, schizophrenia, suicide, autism, migraine, emesis, alcoholism and neurodegenerative disorders.

Serotonin receptors are divided into seven subfamilies, referred to as 5-HT1 through 5-HT7, inclusive. These subfamilies are further divided into subtypes. For example, the 5-HT2 subfamily is divided into three receptor subtypes: 5-HT2A, 5-HT2B, and 5-HT2C. The human 5-HT2C receptor was first isolated and cloned in 1987, and the human 5-HT2A receptor was first isolated and cloned in 1990. These two receptors are thought to be the site of action of hallucinogenic drugs. Additionally, antagonists to the 5-HT2A and 5-HT2C receptors are believed to be useful in treating depression, anxiety, psychosis and eating disorders.

U.S. Patent Number 4,985,352, describes the isolation, characterization, and expression of a functional cDNA clone encoding the entire human 5-HT1C receptor (now known as the 5HT2C receptor). U.S. Patent Number 5,661,0124 describes the isolation, characterization, and expression of a functional cDNA clone encoding the entire human 5-HT2A receptor.

Mutations of the endogenous forms of the rat 5-HT2A and rat 5-HT2C receptors have been reported to lead to constitutive activation of these receptors (5-HT2A: Casey, C. *et al.* (1996) *Society for Neuroscience Abstracts*, 22:699.10, hereinafter "Casey"; 5-HT2C: Herrick-Davis, K., and Teitler, M. (1996) *Society for Neuroscience Abstracts*, 22:699.18, hereinafter "Herrick-Davis 1"; *and* Herrick-Davis, K. *et al.* (1997) *J.Neurochemistry* 69(3): 1138, hereinafter "Herrick-Davis-2"). Casey describes a mutation of the cysteine residue at position 322 of the rat 5-HT2A receptor to lysine (C322K), glutamine (C322Q) and arginine (C322R) which reportedly led to constitutive activation. Herrick-Davis 1 and Herrick-Davis 2 describe

mutations of the serine residue at position 312 of the rat 5-HT2C receptor to phenylalanine (S312F) and lysine (S312K), which reportedly led to constitutive activation.

SUMMARY OF THE INVENTION

The present invention relates to non-endogenous, constitutively activated forms of the human 5-HT2A and human 5-HT2C receptors and various uses of such receptors. Further disclosed are small molecule modulators of these receptors. Most preferably, these modulators have inverse agonist characteristics at the receptor.

More specifically, the present invention discloses nucleic acid molecules and the proteins for three non-endogenous, constitutively activated human serotonin receptors, referred to herein as, AP-1, AP-3, and AP-4. The AP-1 receptor is a constitutively active form of the human 5-HT2C receptor created by an S310K point mutation. The AP-3 receptor is a constitutively active form of the human 5-HT2A receptor whereby the intracellular loop 3 (IC3) portion and the cytoplasmic-tail portion of the endogenous human 5-HT2A receptor have been replaced with the IC3 portion and the cytoplasmic-tail portion of the human 5-HT2C receptor. The AP-4 receptor is a constitutively active form of the human 5-HT2A receptor whereby (1) the region of the intracellular third loop between the proline of the transmembrane 5 region (TM5) and the proline of TM6 of the endogenous human 5-HT2A receptor has been replaced with the corresponding region of the human 5-HT2C receptor (including a S310K point mutation); and (2) the cytoplasmic-tail portion of the endogenous human 5-HT2A receptor has been replaced with the cytoplasmic-tail portion of the endogenous human 5-HT2C receptor.

The invention also provides assays that may be used to directly identify candidate compounds as agonists, partial agonists or inverse agonists to non-endogenous, constitutively activated human serotonin receptors; such candidate compounds can then be utilized in pharmaceutical composition(s) for treatment of diseases and disorders which are related to the human 5-HT2A and/or human 5-HT2C receptors.

These and other aspects of the invention disclosed herein will be set forth in greater detail as the patent disclosure proceeds.

BRIEF DESCRIPTION OF THE DRAWINGS

In the following figures, bold typeface indicates the location of the mutation in the non-endogenous, constitutively activated receptor relative to the corresponding endogenous receptor.

Figure 1 shows a generalized structure of a G protein-coupled receptor with the numbers assigned to the transmembrane helices, the intracellular loops, and the extracellular loops.

Figure 2 schematically shows the active and inactive states for a typical G protein-coupled receptor and the linkage of the active state to the second messenger transduction pathway.

Figure 3a provides the nucleic acid sequence of the endogenous human 5-HT2A receptor (SEQ.ID.NO: 24).

Figure 3b provides the corresponding amino acid sequence of the endogenous human 5-HT2A receptor (SEQ.ID.NO: 25).

Figure 4a provides the nucleic acid sequence of the endogenous human 5-HT2C receptor (SEQ.ID.NO: 26).

Figure 4b provides the corresponding amino acid sequence of the endogenous human 5-HT2C receptor (SEQ.ID.NO: 27).

Figure 5a provides the nucleic acid sequence of a constitutively active form of the human 5-HT2C receptor ("AP-1 cDNA" – SEQ.ID.NO: 28).

Figure 5b provides the corresponding amino acid sequence of the AP-1 cDNA ("AP-1" – SEQ.ID.NO: 29).

Figure 6a provides the nucleic acid sequence of a constitutively active form of the human 5-HT2A receptor whereby the IC3 portion and the cytoplasmic-tail portion of the endogenous 5-HT2A receptor have been replaced with the IC3 portion and the cytoplasmic-tail portion of the human 5-HT2C receptor ("AP-3 cDNA" – SEQ.ID.NO: 30).

Figure 6b provides the corresponding amino acid sequence of the AP-3 cDNA ("AP-3" – SEQ.ID.NO: 31).

Figure 6c provides a schematic representation of AP-3, where the dashed-lines represent the portion obtained from the human 5-HT2C receptor.

Figure 7a provides the nucleic acid sequence of a constitutively active form of the human 5-HT2A receptor whereby (1) the region of the between the proline of TM5 and the proline of TM6 of the endogenous human 5-HT2A receptor has been replaced with the corresponding region of the human 5-HT2C receptor (including a S310K point mutation); and (2) the cytoplasmic-tail portion of the endogenous 5-HT2A receptor has been replaced with the cytoplasmic-tail portion of the endogenous human 5-HT2C receptor ("AP-4 cDNA" – SEQ.ID.NO:32).

Figure 7b provides the corresponding amino acid sequence of the AP-4 cDNA ("AP-4" – SEQ.ID.NO: 33).

Figure 7c provides a schematic representation of the mutated 5-HT2A receptor of Figure 7b where the dashed-lines represent the portion obtained from the human 5-HT2C receptor.

Figure 8 is a representation of the preferred vector, pCMV, used herein.

Figure 9 is a diagram illustrating (1) enhanced [^{35}S]GTPγS binding to membranes prepared from COS cells expressing the endogenous human 5-HT2C receptor in response to serotonin, and (2) inhibition by mianserin using wheatgerm agglutinin scintillation proximity beads. The concentration of [^{35}S]GTPγS was held constant at 0.3 nM, and the concentration of GDP was held at 1 μM. The concentration of the membrane protein was 12.5 μg.

Figure 10 is a diagram showing serotonin stimulation of [^{35}S]GTPγS binding to membranes expressing AP-1 receptors in 293T cells and the inhibition by 30 μM mianserin on Wallac™ scintistrips.

Figure 11 is a diagram showing the effects of protein concentration on [^{35}S]GTPγS binding in membranes prepared from 293T cells transfected with the endogenous human 5-HT2C receptors and AP-1 receptors compared to cells transfected with the control vector (pCMV) alone in the absence (A) and presence (B) of 10 μM serotonin. The radiolableled concentration of [^{35}S]GTPγS was held constant at 0.3 nM, and the GDP concentration was held constant at 1 μM. The assay was performed on 96-well format on Wallac™ scintistrips.

Figure 12 provides bar-graph comparisons of inositol trisphosphate ("IP3") production between the endogenous human 5HT2A receptor and AP-2, a mutated form of the receptor.

Figure 13 provides bar-graph comparisons of inositol trisphosphate ("IP3") production between the endogenous human 5HT2A receptor and AP-4, a mutated form of the receptor.

Figure 14 provides bar graph comparisons of IP3 production between the endogenous human 5-HT2A receptor and AP-3, a mutated form of the receptor.

Figure 15 provides bar-graph comparisons of IP3 production between the endogenous human 5-HT2C receptor and AP-1.

Figures 16A-C provides representative auoradiograms showing displacement of I^{125}-LSD from brain sections by spiperone and compound 116100.

Figure 17 shows in vivo response of animals to 116102 exposure.

DEFINITIONS

The scientific literature that has evolved around receptors has adopted a number of terms to refer to ligands having various effects on receptors. For clarity and consistency, the following definitions will be used throughout this patent document. To the extent that these definitions conflict with other definitions for these terms, the following definitions shall control.

AGONISTS shall mean moieties that activate the intracellular response when they bind to the receptor, or enhance GTP binding to membranes.

AMINO ACID ABBREVIATIONS used herein are set out in Table 1:

TABLE 1

ALANINE	ALA	A
ARGININE	ARG	R
ASPARAGINE	ASN	N
ASPARTIC ACID	ASP	D
CYSTEINE	CYS	C
GLUTAMIC ACID	GLU	E
GLUTAMINE	GLN	Q
GLYCINE	GLY	G
HISTIDINE	HIS	H
ISOLEUCINE	ILE	I
LEUCINE	LEU	L
LYSINE	LYS	K
METHIONINE	MET	M
PHENYLALANINE	PHE	F
PROLINE	PRO	P

SERINE	SER	S
THREONINE	THR	T
TRYPTOPHAN	TRP	W
TYROSINE	TYR	Y
VALINE	VAL	V

PARTIAL AGONISTS shall mean moieties which activate the intracellular response when they bind to the receptor to a lesser degree/extent than do agonists, or enhance GTP binding to membranes to a lesser degree/extent than do agonists.

ANTAGONIST shall mean moieties that competitively bind to the receptor at the same site as the agonists but which do not activate the intracellular response initiated by the active form of the receptor, and can thereby inhibit the intracellular responses by agonists or partial agonists. ANTAGONISTS do not diminish the baseline intracellular response in the absence of an agonist or partial agonist.

CANDIDATE COMPOUND shall mean a molecule (for example, and not limitation, a chemical compound) which is amenable to a screening technique.

COMPOUND EFFICACY shall mean a measurement of the ability of a compound to inhibit or stimulate receptor functionality, as opposed to receptor binding affinity.

CONSTITUTIVELY ACTIVATED RECEPTOR shall mean a receptor subject to constitutive receptor activation.

CONSTITUTIVE RECEPTOR ACTIVATION shall mean stabilization of a receptor in the active state by means other than binding of the receptor with its endogenous ligand or a chemical equivalent thereof.

CONTACT or **CONTACTING** shall mean bringing at least two moieties together, whether in an in vitro system or an in vivo system.

ENDOGENOUS shall mean a material that a mammal naturally produces. ENDOGENOUS in reference to, for example and not limitation, the term "receptor" shall mean that which is naturally produced by a mammal (for example, and not limitation, a human) or a virus.

In contrast, the term **NON-ENDOGENOUS** in this context shall mean that which is not naturally produced by a mammal (for example, and not limitation, a human) or a virus. For

example, and not limitation, a receptor which is not constitutively active in its endogenous form, but when manipulated becomes constitutively active, is most preferably referred to herein as a "non-endogenous, constitutively activated receptor." Both terms can be utilized to describe both "in vivo" and "in vitro" systems. For example, and not a limitation, in a screening approach, the endogenous or non-endogenous receptor may be in reference to an in vitro screening system. As a further example and not limitation, where the genome of a mammal has been manipulated to include a non-endogenous constitutively activated receptor, screening of a candidate compound by means of an in vivo system is viable.

INHIBIT or **INHIBITING**, in relationship to the term "response" shall mean that a response is decreased or prevented in the presence of a compound as opposed to in the absence of the compound.

INVERSE AGONISTS shall mean moieties that bind the endogenous form of the receptor or to the constitutively activated form of the receptor, and which inhibit the baseline intracellular response initiated by the active form of the receptor below the normal base level of activity which is observed in the absence of agonists or partial agonists, or decrease GTP binding to membranes. Preferably, the baseline intracellular response is inhibited in the presence of the inverse agonist by at least 30%, more preferably by at least 50%, and most preferably by at least 75%, as compared with the baseline response in the absence of the inverse agonist.

LIGAND shall mean an endogenous, naturally occurring molecule specific for an endogenous, naturally occurring receptor.

PHARMACEUTICAL COMPOSITION shall mean a composition comprising at least one active ingredient, whereby the composition is amenable to investigation for a specified, efficacious outcome in a mammal (for example, and not limitation, a human). Those of ordinary skill in the art will understand and appreciate the techniques appropriate for determining whether an active ingredient has a desired efficacious outcome based upon the needs of the artisan.

STIMULATE or **STIMULATING**, in relationship to the term "response" shall mean that a response is increased in the presence of a compound as opposed to in the absence of the compound.

DETAILED DESCRIPTION

I. Particularly preferred mutations

For convenience, the sequence information regarding the non-endogenous, constitutively active human 5-HT2A and 5-HT2C receptors are referred to by identifiers as set forth in Table 2:

TABLE 2

IDENTIFIER	RECEPTOR	SEQ.ID.NO:	FIGURE
AP-1 cDNA	5-HT2C	28	5a
AP-1	5-HT2C	29	5b
AP-3 cDNA	5-HT2A	30	6a
AP-3	5-HT2A	31	6b
AP-4 cDNA	5-HT2A	32	7a
AP-4	5-HT2A	33	7b

As will be discussed in greater detail below, a mutation analogous to that reported by Casey (C322K) was utilized in the human 5-HT2A receptor and is referred to herein as AP-2. However, AP-2 did not lead to sufficient constitutive activation to allow for utilization in screening techniques.

II. Introduction

While it is sometimes possible to make predictions as to the effect of nucleic acid manipulation from one species to another, this is not always the case. The results reported by Casey suggest that a point mutation in the rat 5-HT2A receptor evidences constitutive activation of the mutated receptor. Casey reports that the C322K mutation was approximately four fold more active than the native rat 5-HT2A receptor. However, for purposes of a most preferred use, i.e., screening of candidate compounds, this corresponding mutation in the human 5-HT2A receptor had little discernable effect in evidencing constitutive activation of the human receptor. This, of course, creates the reasonable conclusion that the information reported in Herrick-Davis 1 or Herrick-Davis 2 is of limited predictive value relative to the manipulation of the human 5-HT2C receptor. Consequently, the ability to make reasonable predictions about the effects of mutations to the rat 5-HT receptors vis-à-vis the corresponding human receptors is not possible. Nonetheless, this unfortunate lack of reasonable predictability

provides the opportunity for others to discover mutations to the human 5-HT receptors that provide evidence of constitutive activation.

Therefore, the present invention is based upon the desire of defining mutated sequences of the human serotonin receptors 5-HT2A and 5-HT2C whereby such mutated versions of the expressed receptor are constitutively active. These constitutively active receptors allow for, inter alia, screening candidate compounds.

What has been discovered and disclosed herein is that substantial activation of the human 5-HT2A receptor can be obtained by "domain swapping," i.e., by switching the third intracellular domain of the 5-HT2A receptor with the third intracellular domain of the 5-HT2C receptor. Additionally, swapping the cytoplasmic tail of the two receptors further increases the IP3 response. Furthermore, mutation of the serine at position 310 to lysine (S310K) of the human 5-HT2C receptor leads to constitutive activation.

What follows is a most preferred approach to identification of candidate compounds; those in the art will readily appreciate that the particular order of screening approaches, and/or whether or not to utilize certain of these approaches, is a matter of choice. Thus, the order presented below, set for presentational efficiency and for indication of the most preferred approach utilized in screening candidate compounds, is not intended, nor is to be construed, as a limitation on the disclosure, or any claims to follow.

III. Generic G Protein-Coupled Receptor screening assay techniques

When a G protein receptor becomes constitutively active, it binds to a G protein (Gq, Gs, Gi, Go) and stimulates the binding of GTP to the G protein. The G protein then acts as a GTPase and slowly hydrolyzes the GTP to GDP, whereby the receptor, under normal conditions, becomes deactivated. However, constitutively activated receptors continue to exchange GDP to GTP. A non-hydrolyzable analog of GTP, $[^{35}S]GTP\gamma S$, can be used to monitor enhanced binding to membranes which express constitutively activated receptors. It is reported that $[^{35}S]GTP\gamma S$ can be used to monitor G protein coupling to membranes in the absence and presence of ligand. An example of this monitoring, among other examples well-known and available to those in the art, was reported by Traynor and Nahorski in 1995. The preferred use of this assay system is for initial screening of candidate compounds because the system is generically applicable to all G protein-coupled receptors regardless of the particular G protein that interacts with the intracellular domain of the receptor.

IV. Confirmation of G Protein-Coupled Receptor site screening assay techniques

Once candidate compounds are identified using the "generic" G protein-coupled receptor assay (i.e. an assay to select compounds that are agonists, partial agonists, or inverse agonists), further screening to confirm that the compounds have interacted at the receptor site is preferred. For example, a compound identified by the "generic" assay may not bind to the receptor, but may instead merely "uncouple" the G protein from the intracellular domain. Thus, by further screening those candidate compounds, which have been identified using a "generic" assay in an agonist and/or antagonist competitive binding assay, further refinement in the selection process is provided.

Lysergic acid diethylamide (LSD) is a well-known agonist of the 5-HT2A and 5-HT2C receptors, while mesulergine is a well-known antagonist to the 5-HT2C receptor. Accordingly, in most preferred embodiments, an agonist (LSD) and/or antagonist (mesulergine) competitive binding assay(s) is used to further screen those compounds selected from the "generic" assay for confirmation of serotonin receptor binding.

V. Specified G Protein assay techniques

The art-accepted physiologically mediated pathway for the human 5-HT2A and 5-HT2C receptors is via Gq. Intracellular accumulation of IP3 can be used to confirm constitutive activation of these types of Gq coupled receptors (*see* Herrick-Davis-1). As a result, "IP3 accumulation" assays can be used to further screen those compounds selected from an agonist and/or antagonist competitive binding assay.

VI. Pharmaceutical compositions

Candidate compounds selected for further development can be formulated into pharmaceutical compositions using techniques well known to those in the art. Suitable pharmaceutically-acceptable carriers are available to those in the art; for example, see Remington's Pharmaceutical Sciences, 16th Edition, 1980, Mack Publishing Co., (Oslo et al., eds.)

EXAMPLES

The following examples are presented for purposes of elucidation, and not limitation, of the present invention. While specific nucleic acid and amino acid sequences are disclosed herein, those of ordinary skill in the art are credited with the ability to make minor modifications to these sequences while achieving the same or substantially similar results reported below. It is intended that equivalent, non-endogenous, constitutively

activated human serotonin receptor sequences having eighty-five percent (85%) homology, more preferably having ninety percent (90%) homology, and most preferably having ninety-five percent (95%) homology to the disclosed and claimed sequences all fall within the scope of any claims appended hereto.

Example 1

GENERATION OF NON-ENDOGENOUS, CONSTITUTIVELY ACTIVATED HUMAN SEROTONIN RECEPTORS 5-HT2C AND 5-HT2A

A. Construction of constitutively active 5-HT2C receptor cDNA

1. Endogenous Human 5-HT2C

The cDNA encoding endogenous human 5-HT2C receptor was obtained from human brain poly-A^+ RNA by RT-PCR. The 5' and 3' primers were derived from the 5' and 3' untranslated regions and contained the following sequences:

5'-GACCTCGAGGTTGCTTAAGACTGAAGCA-3' (SEQ.ID.NO:1)

5'-ATTTCTAGACATATGTAGCTTGTACCGT-3' (SEQ.ID.NO:2)

PCR was performed using either TaqPlus™ precision polymerase (Stratagene) or rTth™ polymerase (Perkin Elmer) with the buffer systems provided by the manufacturers, 0.25 μM of each primer, and 0.2 mM of each of the four (4) nucleotides. The cycle condition was 30 cycles of 94°C for 1 minute, 57 °C for 1 minute and 72 °C for 2 minutes. The 1.5 kb PCR fragment was digested with Xho I and Xba I and subcloned into the Sal I-Xba I site of pBluescript.

The derived cDNA clones were fully sequenced and found to correspond to published sequences.

2. AP-1 cDNA

The cDNA containing a S310K mutation (AP-1 cDNA) in the third intracellular loop of the human 5-HT2C receptor was constructed by replacing the Sty I restriction fragment containing amino acid 310 with synthetic double stranded oligonucleotides encoding the desired mutation. The sense strand sequence utilized had the following sequence:

5'-
CTAGGGGCACCATGCAGGCTATCAACAATGAAAGAAAAGCTAAGAAAGTC-3'
(SEQ. ID.NO: 3)

and the antisense strand sequence utilized had the following sequence:

5'-CAAGGACTTTCTTAGCTTTTCTTTCATTGTTGATAGCCTGCATGGT
GCCC-3' (SEQ. ID. NO: 4).

B. Construction of constitutively active 5-HT2A receptor cDNA

1. Endogenous Human 5-HT2A

The cDNA encoding endogenous human 5-HT2A receptor was obtained by RT-PCR using human brain poly-A^+ RNA; a 5' primer from the 5' untranslated region with a Xho I restriction site:

5'-GACCTCGAGTCCTTCTACACCTCATC-3' (SEQ.ID.NO:5)

and a 3' primer from the 3' untranslated region containing an Xba I site:

5'-TGCTCTAGATTCCAGATAGGTGAAAA CTTG-3' (SEQ.ID.NO:6).

PCR was performed using either TaqPlus™ precision polymerase (Stratagene) or rTth™ polymerase (Perkin Elmer) with the buffer systems provided by the manufacturers, 0.25 μM of each primer, and 0.2 mM of each of the four (4) nucleotides. The cycle condition was 30 cycles of 94°C for 1 minute, 57 °C for 1 minute and 72 °C for 2 minutes. The 1.5 kb PCR fragment was digested with Xba I and subcloned into the Eco RV-Xba I site of pBluescript.

The resulting cDNA clones were fully sequenced and found to encode two amino acid changes from the published sequences. The first change is a T25N mutation in the N-terminal extracellular domain and the second change is an H452Y mutation. These mutations are likely to represent sequence polymorphisms rather than PCR errors since the cDNA clones having the same two mutations were derived from two independent PCR procedures using Taq polymerase from two different commercial sources (TaqPlus™ Stratagene and rTth™ Perkin Elmer).

2. Human 5-HT2A (C322K; AP-2)

The cDNA containing the point mutation C322K in the third intracellular loop was constructed by using the Sph I restriction enzyme site, which encompasses amino acid 322. For the PCR procedure, a primer containing the C322K mutation:

5'-CAAAGAAAGTACTGGGCATCGTCTTCTTCCT-3' (SEQ.ID.NO:7)

was used along with the primer from the 3' untranslated region set forth above as SEQ.ID.NO:6. The resulting PCR fragment was then used to replace the 3' end of the wild type 5-HT2A cDNA by the T4 polymerase blunted Sph I site. PCR was performed using pfu polymerase (Stratagene) with the buffer system provided by the manufacturer and 10% DMSO, 0.25 mM of each primer, 0.5mM of each of the 4 nucleotides. The cycle conditions were 25 cycles of 94°C for 1 minute, 60°C for 1 minute and 72°C for 1 minute.

3. AP-3 cDNA

The human 5-HT2A cDNA with intracellular loop 3 (IC3) or IC3 and cytoplasmic tail replaced by the corresponding human 5-HT2C cDNA was constructed using PCR-based mutagenesis.

(a) Replacement of IC3 Loop

The IC3 loop of human 5-HT2A cDNA was first replaced with the corresponding human 5-HT2C cDNA. Two separate PCR procedures were performed to generate the two fragments, Fragment A and Fragment B, that fuse the 5-HT2C IC3 loop to the transmembrane 6 (TM6) of 5-HT2A. The 237 bp PCR fragment, Fragment A, containing 5-HT2C IC3 and the initial 13 bp of 5-HT2A TM6 was amplified by using the following primers:

5'-CCGCTCGAGTACTGCGCCGACAAGCTTTGAT-3' (SEQ.ID.NO:8)

5'-CGATGCCCAGCACTTTCGAAGCTTTTCTTTCATTGTTG3'(SEQ.ID.NO:9)

The template used was human 5-HT2C cDNA.

The 529 bp PCR fragment, Fragment B, containing the C-terminal 13 bp of IC3 from 5-HT2C and the C-terminal of 5-HT2A starting at beginning of TM6, was amplified by using the following primers:

5'-AAAAGCTTCGAAAGTGCTGGGCATCGTCTTCTTCCT-3' (SEQ.ID.NO:10)

5'-TGCTCTAGATTCCAGATAGGTGAAAACTTG-3' (SEQ.ID.NO: 11)

The template used was human 5-HT2A cDNA.

Second round PCR was performed using Fragment A and Fragment B as co-templates with SEQ.ID.NO:8 and SEQ.ID.NO:11 (it is noted that the sequences for SEQ.ID.NOS.: 6 and 11 are the same) as primers. The resulting 740 bp PCR fragment, Fragment C, contained the IC3 loop of human 5-HT2C fused to TM6 through the end of the cytoplasmic tail of human 5-HT2A. PCR was performed using pfu™ polymerase (Stratagene) with the buffer system provided by the manufacturer, and 10% DMSO, 0.25 mM of each primer, and 0.5 mM of each of the four (4) nucleotides. The cycle conditions were 25 cycles of 94 °C for 1 minute, 57 °C (1st round PCR) or 60 °C (2nd round PCR) for 1 minute, and 72 °C for 1 minute (1st round PCR) or 90 seconds. (2nd round PCR).

To generate a PCR fragment containing a fusion junction between the human 5-HT2A TM5 and the IC3 loop of 5-HT2C, four (4) primers were used. The two external primers, derived from human 5-HT2A, had the following sequences:

5'-CGTGTCTCTCCTTACTTCA-3' (SEQ.ID.NO:12)

The other primer used was SEQ.ID.NO.6 (see note above regarding SEQ.ID.NOS. 6 and 11). The first internal primer utilized was an antisense strand containing the initial 13 bp of IC3 of 5-HT2C followed by the terminal 23 bp derived from TM5 of 5-HT2A:

5'-TCGGCGCAGTACTTTGATAGTTAGAAAGTAGGTGAT-3' (SEQ.ID.NO:13)

The second internal primer was a sense strand containing the terminal 14 bp derived from TM5 of 5-HT2A followed by the initial 24 bp derived from IC3 of 5-HT2C:

5'-TTCTAACTATCAAAGTACTGCGCCGACAAGCTTTGATG-3' (SEQ.ID.NO:14).

PCR was performed using endogenous human 5-HT2A and a co-template, Fragment C, in a 50 ml reaction volume containing 1X pfu buffer, 10% DMSO, 0.5 mM of each of the four (4) nucleotides, 0.25 mM of each external primer (SEQ.ID.NOS. 11 and 12), 0.06 mM of each internal primer (SEQ.ID.NOS. 13 and 14) and 1.9 units of pfu polymerase (Stratagene). The cycle conditions were 25 cycles of 94°C for 1 minute, 52°C for 1 minute and 72 °C for 2 minutes and 10 seconds. The 1.3 kb PCR product was then gel purified and digested with Pst I and Eco RI. The resulting 1 kb PstI-Eco RI fragment was used to replace the corresponding fragment in the endogenous human 5-HT2A sequence to generate the mutant 5-HT2A sequence encoding the IC3 loop of 5-HT2C.

(b) Replacement of the cytoplasmic tail

To replace the cytoplasmic tail of 5-HT2A with that of 5-HT2C, PCR was performed using a sense primer containing the C-terminal 22 bp of TM7 of endogenous human 5-HT2A followed by the initial 21 bp of the cytoplasmic tail of endogenous human 5-HT2C:

5'-TTCAGCAGTCAACCCACTAGTCTATACTCTGTTCAACAAAATT-3' (SEQ.ID.NO:15)

The antisense primer was derived from the 3' untranslated region of endogenous human 5-HT2C:

5'-ATTTCTAGACATATGTAGCTTGTACCGT-3' (SEQ.ID.NO:16).

The resulting PCR fragment, Fragment D, contained the last 22 bp of endogenous human 5-HT2A TM7 fused to the cytoplasmic tail of endogenous human 5-HT2C. Second round PCR was performed using Fragment D and the co-template was endogenous human 5-HT2A that was previously digested with Acc I to avoid undesired amplification. The antisense primer used was SEQ.ID.NO:16 (the sequences for SEQ.ID.NOS. 16 and 2 are the same) and the sense primer used was derived from endogenous human 5-HT2A:

5'-ATCACCTACTTTCTAACTA-3' (SEQ.ID.NO:17).

PCR conditions were as set forth in Example 1B3.(a) for the first round PCR, except that the annealing temperature was 48 °C and the extension time was 90 seconds. The resulting 710 bp PCR product was digested with Apa I and Xba I and used to replace the corresponding Apa I-Xba I fragment of either (a) endogenous human 5-HT2A, or (b) 5-HT2A with 2C IC3 to generate (a) endogenous human 5-HT2A with endogenous human 5-HT2C cytoplasmic tail and (b) AP-3, respectively.

4. *AP-4 cDNA*

This mutant was created by replacement of the region of endogenous human 5-HT2A from amino acid 247, the middle of TM5 right after Pro^{246}, to amino acid 337, the middle of TM6 just before Pro^{338}, by the corresponding region of AP-1 cDNA. For convenience, the junction in TM5 is referred to as the "2A-2C junction," and the junction in TM6 is referred to as the "2C-2A junction."

Three PCR fragments containing the desired hybrid junctions were generated. The 5' fragment of 561 bp containing the 2A-2C junction in TM5 was generated by PCR using endogenous human 5-HT2A as template, SEQ.ID.NO:12 as the sense primer, and the antisense primer was derived from 13 bp of 5-HT2C followed by 20 bp of 5-HT2A sequence:

5'-CCATAATCGTCAGGGGAATGAAAAATGACACAA-3' (SEQ.ID.NO:18)

The middle fragment of the 323 bp contains endogenous human 5-HT2C sequence derived from the middle of TM5 to the middle of TM6, flanked by 13 bp of 5-HT2A sequences from the 2A-2C junction and the 2C-2A junction. This middle fragment was generated by using AP-1 cDNA as a template, a sense primer containing 13 bp of 5-HT2A followed by 20 bp of 5-HT2C sequences across the 2A-2C junction and having the sequence:

5'-ATTTTTCATTCCCCTGACGATTATGGTGATTAC-3' (SEQ.ID.NO:19);

and an antisense primer containing 13 bp of 5-HT2A followed by 20 bp of 5-HT2C sequences across the 2C-2A junction and having the sequence:

5'-TGATGAAGAAAGGGCACCACATGATCAGAAACA-3' (SEQ.ID.NO:20).

The 3' fragment of 487 bp containing the 2C-2A junction was generated by PCR using endogenous human 5-HT2A as a template and a sense primer having the following sequence from the 2C-2A junction:

5'-GATCATGTGGTGCCCTTTCTTCATCACAAACAT-3' (SEQ.ID.NO:21)

and the antisense primer was SEQ.ID.NO:6 see note above regarding SEQ.ID.NOS. 6 and 11).

Two second round PCR reactions were performed separately to link the 5' and middle fragment (5'M PCR) and the middle and 3' fragment (M3' PCR). The 5'M PCR co-template used was the 5' and middle PCR fragment as described above, the sense primer was SEQ.ID.NO:12 and the antisense primer was SEQ.ID.NO:20. The 5'M PCR procedure resulted in an 857 bp PCR fragment.

The M3' PCR used the middle and M3' PCR fragment described above as the co-template, SEQ.ID.NO: 19 as the sense primer and SEQ.ID.NO:6 (see note above regarding SEQ.ID.NOS. 6 and 11) as the antisense primer, and generated a 784 bp amplification product. The final round of PCR was performed using the 857 bp and 784 bp fragments from the second round PCR as the co-template, and SEQ.ID.NO:12 and SEQ.ID.NO: 6 (see note above regarding SEQ.ID.NOS. 6 and 11) as the sense and the antisense primer, respectively. The 1.32 kb amplification product from the final round of PCR was digested with Pst I and Eco RI. Then resulting 1 kb Pst I-Eco RI fragment was used to replace the corresponding fragment of the endogenous human 5-HT2A to generate mutant 5-HT2A with 5-HT2C: C310K/IC3. The Apa I-Xba fragment of AP3 was used to replace the corresponding fragment in mutant 5-HT2A with 5-HT2C: C310K/IC3 to generate AP4.

Example 2

RECEPTOR EXPRESSION

A. pCMV

Although a variety of expression vectors are available to those in the art, for purposes of utilization for both the endogenous and non-endogenous receptors discussed herein, it is most preferred that the vector utilized be pCMV. This vector was deposited with the American Type Culture Collection (ATCC) on October 13, 1998 (10801 University Blvd., Manassas, VA 20110-2209 USA) under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. The DNA was tested by the ATCC and determined to be viable. The ATCC has assigned the following deposit number to pCMV: ATCC #203351. *See* Figure 8.

B. Transfection procedure

For the generic assay ([^{35}S]GTPγS; Example 3) and the antagonist binding assay (mesulergine; Example 4), transfection of COS-7 or 293T cells was accomplished using the following protocol.

On day one, $5X10^6$ COS-7 cells or $1X10^7$ 293T cells per 150mm plate were plated out. On day two, two reaction tubes were prepared (the proportions to follow for each tube are per plate): tube A was prepared by mixing 20μg DNA (e.g., pCMV vector; pCMV vector AP-1 cDNA, etc.) in 1.2ml serum free DMEM (Irvine Scientific, Irvine, CA); tube B was prepared by mixing 120μl lipofectamine (Gibco BRL) in 1.2ml serum free DMEM. Tubes A and B were then admixed by inversions (several times), followed by incubation at room temperature for 30-45min. The admixture is referred to as the "transfection mixture". Plated COS-7 cells were washed with 1X PBS, followed by addition of 10ml serum free DMEM. 2.4ml of the transfection mixture was then added to the cells, followed by incubation for 4hrs at 37°C/5% CO_2. The transfection mixture was then removed by aspiration, followed by the addition of 25ml of DMEM/10% Fetal Bovine Serum. Cells were then incubated at 37°C/5% CO_2. After 72hr incubation, cells were then harvested and utilized for analysis.

Example 3

GTP Membrane Binding Scintillation Proximity Assay

The advantages of using [^{35}S]GTPγS binding to measure constitutive activation are that: (a) [^{35}S]GTPγS binding is generically applicable to all G protein-coupled receptors; and (b) [^{35}S]GTPγS binding is proximal at the membrane surface, thereby making it less likely to pick-up molecules which affect the intracellular cascade. The assay utilizes the ability of G protein-coupled receptors to stimulate [^{35}S]GTPγS binding to membranes expressing the relevant receptors. Therefore, the assay may be used to directly screen compounds at the disclosed serotonin receptors.

Figure 9 demonstrates the utility of a scintillation proximity assay to monitor the binding of [^{35}S]GTPγS to membranes expressing the endogenous human 5-HT2C receptor expressed in COS cells. In brief, the assay was incubated in 20 mM HEPES, pH 7.4, binding buffer with 0.3 nM [^{35}S]GTPγS and 12.5 μg membrane protein and 1 μM GDP for 30 minutes. Wheatgerm agglutinin beads (25 μl; Amersham) were then added and the mixture was incubated for another 30 minutes at room temperature. The tubes were then centrifuged at 1500

x g for 5 minutes at room temperature and then counted in a scintillation counter. As shown in Figure 9, serotonin, which as the endogenous ligand activates the 5-HT2C receptor, stimulated [^{35}S]GTPγS binding to the membranes in a concentration dependant manner. The stimulated binding was completely inhibited by 30 μM mianserin, a compound considered as a classical 5-HT2C antagonist, but also known as a 5-HT2C inverse agonist.

Although this assay measures agonist-induced binding of [^{35}S]GTPγS to membranes and can be routinely used to measure constitutive activity of receptors, the present cost of wheatgerm agglutinin beads may be prohibitive. A less costly but equally applicable alternative also meets the needs of large-scale screening. Flash plates and Wallac™ scintistrips may be used to format a high throughput [^{35}S]GTPγS binding assay. This technique allows one to monitor the tritiated ligand binding to the receptor while simultaneously monitoring the efficacy via [^{35}S]GTPγS binding. This is possible because the Wallac™ beta counter can switch energy windows to analyze both tritium and ^{35}S-labeled probes.

Also, this assay may be used for detecting of other types of membrane activation events that result in receptor activation. For example, the assay may be used to monitor ^{32}P phosphorylation of a variety of receptors (including G protein-coupled and tyrosine kinase receptors). When the membranes are centrifuged to the bottom of the well, the bound [^{35}S]GTPγS or the ^{32}P-phosphorylated receptor will activate the scintillant coated on the wells. Use of Scinti® strips (Wallac™) demonstrate this principle. Additionally, this assay may be used for measuring ligand binding to receptors using radiolabeled ligands. In a similar manner, the radiolabeled bound ligand is centrifuged to the bottom of the well and activates the scintillant. The [^{35}S]GTPγS assay results parallel the results obtained in traditional second messenger assays of receptors.

As shown in Figure 10, serotonin stimulates the binding of [^{35}S]GTPγS to the endogenous human 5-HT2C receptor, while mianserin inhibits this response. Furthermore, mianserin acts as a partial inverse agonist by inhibiting the basal constitutive binding of [^{35}S]GTPγS to membranes expressing the endogenous human 5-HT2C receptor. As expected, there is no agonist response in the absence of GDP since there is no GDP present to exchange for [^{35}S]GTPγS. Not only does this assay system demonstrate the response of the native 5-HT2C receptor, but it also measures the constitutive activation of other receptors.

Figure 11A and Figure 11B demonstrate the enhanced binding of [^{35}S]GTPγS to membranes prepared from 293T cells expressing the control vector alone, the native human 5-HT2C receptor or the AP-1 receptor. The total protein concentration used in the assay affects the total amount of [^{35}S]GTPγS binding for each receptor. The c.p.m. differential between the CMV transfected and the constitutively active mutant receptor increased from approximately 1000 c.p.m at 10 μg/well to approximately 6-8000 c.p.m. at 75 μg/well protein concentration, as shown in Figure 11.

The AP-1 receptor showed the highest level of constitutive activation followed by the wild type receptor, which also showed enhanced [^{35}S]GTPγS binding above basal. This is consistent with the ability of the endogenous human 5-HT2C receptor to accumulate intracellular IP3 in the absence of 5HT stimulation (Example 5) and is also consistent with published data claiming that the endogenous human 5-HT2C receptor has a high natural basal activity. Therefore, the AP-1 receptor demonstrates that constitutive activity may be measured by proximal [^{35}S]GTPγS binding events at the membrane interface.

Example 4

SEROTONIN RECEPTOR AGONIST/ANTAGONIST COMPETITIVE BINDING ASSAY

Membranes were prepared from transfected COS-7 cells (*see* Example 2) by homogenization in 20 mM HEPES and 10 mM EDTA , pH 7.4 and centrifuged at 49,000 x g for 15 min. The pellet was resuspended in 20 mM HEPES and 0.1 mM EDTA, pH 7.4, homogenized for 10 sec. using polytron homogenizer (Brinkman) at 5000 rpm and centrifuged at 49,000 x g for 15 min. The final pellet was resuspended in 20 mM HEPES and 10 mM $MgCl_2$, pH 7.4, homogenized for 10 sec. using polytron homogenizer (Brinkman) at 5000 rpm.

Assays were performed in triplicate 200μl volumes in 96 well plates. Assay buffer (20 mM HEPES and 10 mM $MgCl_2$, pH 7.4) was used to dilute membranes, ^{3}H-LSD, ^{3}H-mesulergine, serotonin (used to define non-specific for LSD binding) and mianserin (used to define non-specific for mesulergine binding). Final assay concentrations consisted of 1nM ^{3}H-LSD or 1nM ^{3}H-mesulergine, 50μg membrane protein and 100μm serotonin or mianserin. LSD assays were incubated for 1 hr at 37° C, while mesulergine assays were incubated for 1 hr at room temperature. Assays were terminated by rapid filtration onto Wallac Filtermat Type B with ice cold binding buffer using Skatron cell harvester. The radioactivity was determined in a Wallac 1205 BetaPlate counter.

Example 5

INTRACELLULAR IP3 ACCUMULATION ASSAY

For the IP3 accumulation assay, a transfection protocol different from the protocol set forth in Example 2 was utilized. In the following example, the protocols used for days 1-3 were slightly different for the data generated for Figures 12 and 14 and for Figures 13 and 15; the protocol for day 4 was the same for all conditions.

A. COS-7 and 293 Cells

On day one, COS-7 cells or 293 cells were plated onto 24 well plates, usually $1x10^5$ cells/well or $2x10^5$ cells/well, respectively. On day two, the cells were transfected by first mixing 0.25 μg DNA (*see* Example 2) in 50 μl serum-free DMEM/well and then 2 μl lipofectamine in 50 μl serum-free DMEM/well. The solutions ("transfection media") were gently mixed and incubated for 15-30 minutes at room temperature. The cells were washed with 0.5 ml PBS and then 400 μl of serum free media was mixed with the transfection media and added to the cells. The cells were then incubated for 3-4 hours at 37°C/5%CO_2. Then the transfection media was removed and replaced with 1ml/well of regular growth media. On day 3, the media was removed and the cells were washed with 0.5 ml PBS. Then 0.5 ml inositol-free/serum-free media (GIBCO BRL) was added to each well with 0.25 μCi of ^{3}H-myo-inositol/well and the cells were incubated for 16-18 hours overnight at 37°C/5%CO_2. Protocol A.

B. 293 Cells

On day one, $1x10^7$ 293 cells per 150mm plate were plated out. On day two, two reaction tubes were prepared (the proportions to follow for each tube are per plate): tube A was prepared by mixing 20μg DNA (e.g., pCMV vector; pCMV vector AP-1 cDNA, etc.) in 1.2ml serum free DMEM (Irvine Scientific, Irvine, CA); tube B was prepared by mixing 120μl lipofectamine (Gibco BRL) in 1.2ml serum free DMEM. Tubes A and B were then admixed by inversions (several times), followed by incubation at room temperature for 30-45min. The admixture is referred to as the "transfection mixture". Plated 293 cells were washed with 1XPBS, followed by addition of 10ml serum free DMEM. 2.4ml of the transfection mixture was then added to the cells, followed by incubation for 4hrs at 37°C/5% CO_2. On day 3, cells were trypsinized and counted, followed by plating of $1x10^6$ cells/well (poly D-lysine treated

12-well plates). Cells were permitted to adhere to the wells, followed by one wash with 1xPBS. Thereafter, 0.5 μCi ^{3}H-inositol in 1ml inositol-free DMEM was added per well. Protocol B.

On day 4, the cells were washed with 0.5 ml PBS and then 0.45 ml of assay medium was added containing inositol-free/serum free media, 10 μM pargyline, 10 mM lithium chloride, or 0.4 ml of assay medium and 50 ul of 10x ketanserin (ket) to a final concentration of 10μM. The cells were then incubated for 30 minutes at 37°C. Then the cells were washed with 0.5 ml PBS and 200 ul of fresh/icecold stop solution (1M KOH; 18 mM Na-borate; 3.8 mM EDTA) was added/well. The solution was kept on ice for 5-10 minutes or until the cells were lysed and then neutralized by 200 μl of fresh/ice cold neutralization sol. (7.5 % HCL). The lysate was then transferred into 1.5 ml micro-centrifuge tubes and 1 ml of chloroform/methanol (1:2) was added/tube. The solution was vortexed for 15 seconds and the upper phase was applied to a Biorad AG1-X8 anion exchange resin (100-200 mesh). The resin was washed with water and 0.9 ml of the upper phase was loaded onto the column. The column was washed with 10 mls of 5 mM myo-inositol and 10 ml of 5 mM Na-borate/60mM Na-formate. The inositol trisphosphates were eluted into scintillation vials containing 10 ml of scintillation cocktail with 2 ml of 0.1 M formic acid/ 1 M ammonium formate. The columns were regenerated by washing with 10 ml of 0.1 M formic acid/3M ammonium formate and rinsed twice with dd H_2O and stored at room temperature in water. Results are discussed below.

Figure 12 is an illustration of IP3 production from the human 5-HT2A receptor which was mutated using the same point mutation as set forth in Casey, which rendered the rat receptor constitutively active. The results represented in Figure 12, support the position that when the point mutation shown to activate the rat receptor is introduced into the human receptor, little activation of the receptor is obtained that would allow for appropriate screening of candidate compounds, with the response being only moderately above that of the endogenous human 5-HT2A receptor. Generally, a response of at least 2X above that of the endogenous response is preferred.

Figure 13 provides an illustration comparing IP3 production from endogenous 5-HT2A receptor and the AP4 mutation. The results illustrated in Figure 13 support the position that when the novel mutation disclosed herein is utilized, a robust response of constitutive IP3 accumulation is obtained (e.g., over 2X that of the endogenous receptor).

Figure 14 provides an illustration of IP3 production from AP3. The results illustrated in Figure 14 support the position that when the novel mutation disclosed herein is utilized, a robust response of constitutive IP3 accumulation is obtained.

Figure 15 provides bar-graph comparisons of IP3 accumulation between endogenous human 5-HT2C receptor and AP-1. Note that the endogenous receptor has a high degree of natural constitutive activity relative to the control CMV transfected cells (i.e., the endogenous receptor appears to be constitutively activated).

Example 6

SCREENING OF COMPOUNDS KNOWN TO HAVE 5-HT2C ANTAGONIST ACTIVITY AGAINST NON-ENDOGENOUS, CONSTITUTIVELY ACTIVATED HUMAN SEROTONIN RECEPTOR: AP-1

A final concentration of 12.5 μg membranes prepared from COS7 cells (*see* Example 2) transiently expressing constitutively active mutant human 5HT2C receptor AP-1 were incubated with binding buffer (20 mM HEPES, pH 7.4, 100 mM NaCl, 20 mM $MgCl_2.6H_2O$, 0.2% saponin, and 0.2 mM ascobate), GDP(1μM) and compound in a 96-well plate format for a period of 60 minutes at ambient room temperature. Plates were then centrifuged at 4,000 rpm for 15 minutes followed by aspiration of the reaction mixture and counting for 1 minute in a Wallac™ MicroBeta plate scintillation counter. A series of compounds known to possess reported 5HT2C antagonist activity were determined to be active in the [^{35}S]GTPγS binding assay using AP-1. IC_{50} determinations were made for these commercially available compounds (RBI, Natick, MA). Results are summarized in Table 3. For each determination, eight concentrations of test compounds were tested in triplicate. The negative control in these experiments consisted of AP-1 receptor without test compound addition, and the positive control consisted of 12.5 μg/well of COS7 cell membranes expressing the CMV promoter without expressed AP-1 receptor.

TABLE 3

Test Compound	Known Pharmacology	IC_{50} (nM) in GTP-γ-[^{35}S] Assay
Metergoline	5HT2/1C antagonist	32.0

Mesulergine	5HT2/1C antagonist	21.2
Methysergide	5HT2/1C antagonist	6.1
Methiothepin	5HT1 antagonist	20.4
Normethylclozapin	5HT2/1C antagonist	21.4
Fluoxetine	5HT reuptake inhibitor	114.0
Ritanserin	5HT2/1C antagonist	19.4

The IC_{50} results confirm that the seven tested compounds showed antagonist activity at the AP-1 receptor.

Example 7

SCREENING OF CANDIDATE COMPOUNDS AGAINST NON-ENDOGENOUS, CONSTITUTIVELY ACTIVATED HUMAN SEROTONIN RECEPTORS:AP-1

Approximately 5,500 candidate compounds (Tripos, Inc., St. Louis, MO) were screened using the assay protocol of Example 3 (with AP-1 mutant receptor) for identification as inverse agonists against the receptor; for this assay, an arbitrary cut-off of at least 50% inhibition was established for identification of inverse agonists. Approximately 120 of these compounds evidenced at least 50% inhibition of $[^{35}S]GTP\gamma S$ binding at 10 μM candidate compound (data not shown).

Example 8

SCREENING OF SELECTED COMPOUNDS TO CONFIRM RECEPTOR BINDING: AP-1

The candidate compounds identified from Example 7 were then screened using the assay protocol of Example 4 (mesulergine), using the AP-1 mutant receptor. IC_{50} (nM) values were determined; five of the nearly 120 compounds of Example 7 were determined to have potent binding affinity for the receptor. Results are summarized in Table 4.

Table 4

Candidate Compound	IC_{50} (nM) in Mesulergine Assay
102461	205.0
102788	46.5
100341	209.0
100431	147.0
103487	1,810.0

Example 9a

GENERAL SCREENING PARADIGM: SELECTION OF PRE-CLINICAL CANDIDATE LEADS

The "primary" screen designed to directly identify human $5HT_{2A}/5HT_{2C}$ receptor inverse agonists consisted of a membrane-based GTPγS binding assay utilizing membranes prepared from COS7 cells transiently transfected with AP-1 human receptor. Candidate compounds (10μM final assay concentration) directly identified as inhibiting receptor-mediated increases in GTPγS binding by greater than 50-75% (arbitrary cut-off value) were considered active "hits". Primary assay hits were then re-tested in the same assay to reconfirm their inverse agonist activity. If primary assay hits were reconfirmed active (50% or greater inhibition), and therefore directly identified as, *e.g.*, an inverse agonist, one of two approaches were available: (a) so-called "directed libraries" could be created, i.e., additional candidate compounds were synthesized based upon the structures of the reconfirmed hits (geared towards, e.g., improvement in the characteristics of the compounds) whereby the directed library compounds were then evaluated for the ability to compete for radioligand binding to both mutant 5HT2C (AP-1) and endogenous 5HT2A receptors, or (b) the reconfirmed hits were then evaluated for the ability to compete for radioligand binding to both mutant 5HT2C (AP-1) and endogenous 5HT2A receptors. Thus, when approach (a) was used, because these directed library candidate compounds were based upon the structures of compounds that were directly identified from the

membrane-based GTPγS binding assay, the directed library compounds were not re-tested in the membrane-based GTPγS binding assay but rather were then confirmed via the radioligand binding analysis. The radioligand binding analysis tests were initially performed at 10μM test compound in triplicate and if the compound inhibited radiolabeled binding by 50% or more, the analysis was followed by eight concentration competition curves to determine Ki values. The last step in secondary assay evaluation was to determine if test compounds were capable of inhibiting AP-3 receptor-mediated accumulation of inositol phosphates (e.g., IP_3). This final assay confirms that the directly identified compounds retained inverse agonist properties.

Example 9b

CONSTITUTIVELY ACTIVATED HUMAN 5HT2C RECEPTOR (AP-1) MEDIATED FACILITATION OF GTPγS BINDING TO COS7 MEMBRANES

This protocol is substantially the same as set forth above in Example 6.

Primary screening assays measuring GTPγS binding to membranes prepared from COS7 cells transiently transfected with human mutated 5HT2C receptor (AP-1) were used to directly identify inverse agonists in screening libraries (Tripos, Inc.). Candidate compound screens were performed in a total assay volume of 200μl using scintillant-coated Wallac Scintistrip™ plates. The primary assay was comprised of the following chemicals (at indicated final assay concentrations): 20 mM HEPES, pH 7.4, 100 mM NaCl, 20 mM $MgCl_2$, 0.2% saponin, 0.2 mM ascorbic acid, 1μM GDP, 0.3 nM $GTP\gamma^{35}S$, and 12.5 μg of the above defined membranes. Incubations were performed for 60 minutes at ambient room temperature. The binding assay incubation was terminated by centrifugation of assay plates at 4,000 rpm for 15 minutes, followed by rapid aspiration of the reaction mixture and counting in a Wallac MicroBeta™ scintillation counter.

Primary screening of candidate compounds initially involved testing of 72 test compounds per assay plate (96-well plates were utilized), at a final assay concentration of 10μM candidate compound, in single replicates. A total of sixteen wells of each plate were dedicated for an eight concentration clozapine (a confirmed 5HT2C/2A inverse agonist) dose response curve (duplicate determinations at each concentration). Finally, a total of five assay wells of each plate were dedicated to define the negative control (AP-1 receptor

expressing membranes without addition of candidate compounds) and three wells from each plate to define the positive control (membranes without AP-1 receptor).

Reconfirmation experiments involve re-testing candidate compounds in the same assay described above, except that candidate compounds were evaluated in triplicate, thus allowing evaluation of 24 compounds per 96-well assay plate. Similar to the primary assay plates, an eight concentration clozapine dose response curve (duplicate determinations at each concentration) and the same negative and positive control wells were also included within each 96-well plate.

Example 9c(1)

COMPETITION STUDIES
MUTATED HUMAN 5HT2C RECEPTOR (AP-1)

Radioligand binding competition experiments were performed in a total assay volume of 200µl using standard 96-well microtiter plates. The final assay ingredients consisted of assay buffer (20mM HEPES and 10mM $MgCl_2$), 1nM [^{3}H]mesulergine, and 50µg of membranes (COS7 with AP-1 as defined above). Nonspecific [^{3}H]mesulergine binding was defined in the presence of 100µM mianserin. Incubations were performed for 1 hour at 37°C. Receptor bound radioligand was resolved from free radioligand by rapid filtration of the assay mixture over a Wallac Filtermat™ Type B filter, followed by washing with ice-cold assay buffer using a Skatron™ cell harvester. Radioactivity was counted using a Wallac 1205 BetaPlate™ counter. Each assay plate contained five negative control wells (membranes expressing receptor and no candidate compound addition) and three positive control wells (each containing 100µM mianserin). For one concentration tests, candidate compounds were diluted into assay buffer and screened at a final concentration of 10µM, in triplicate. For IC_{50} determinations, candidate compounds were diluted in assay buffer and eight different concentrations were evaluated, in triplicate. A total of 16 wells were designated for an eight concentration mianserin dose response curve evaluation for both assays.

Example 9c(2)

COMPETITION STUDIES

WILD TYPE HUMAN 5HT2A RECEPTOR

Radioligand binding competition experiments were performed in a total assay volume of 200µl using standard 96-well microtiter plates. The final assay ingredients comprised assay buffer (20mM HEPES and 10mM $MgCl_2$), 1nM [^{3}H]LSD, and 50µg of the above-defined membranes (COS7 with AP-1). Nonspecific [^{3}H]LSD binding was defined in the presence of 100µM serotonin. Incubations were performed for 1 hour at 37°C. Receptor bound radioligand was resolved from free radioligand by rapid filtration of the assay mixture over a Wallac Filtermat™ Type B filter, followed by washing with ice-cold assay buffer using a Skatron™ cell harvester. Radioactivity was counted using a Wallac 1205 BetaPlate™ counter. Each assay plate contained five negative control wells (membranes expressing receptor and no candidate compound addition) and three positive control wells (containing 100µM mianserin). For one concentration tests, candidate compounds were diluted into assay buffer and screened at a final concentration of 10µM in triplicate. For IC_{50} determinations, candidate compounds were diluted in assay buffer and eight different concentrations were evaluated in triplicate. A total of 16 wells were designated for an eight concentration serotonin dose response curve evaluation for both assays.

Example 9d

RECEPTOR-MEDIATED INOSITOL PHOSPHATE ACCUMULATION

Candidate compound identified in the assays of Examples 9a-9c were then evaluated for inositol phosphate accumulation, following the protocol of Example 5 (COS7 cells expressing human mutated 5HT2A receptor, AP-3), modified as follows: tube A was prepared by mixing 16 µg DNA (e.g., pCMV vector; pCMV vector AP-1 cDNA, etc.) in 1.0ml serum free DMEM (Irvine Scientific, Irvine, CA); tube B was prepared by mixing 60µl lipofectamine (Gibco BRL) in 1.0 ml serum free DMEM. Tubes A and B were then admixed by inversions (several times), followed by incubation at room temperature for 30 min. The admixture is referred to as the "transfection mixture". Plated 293 cells were washed with 10 ml Serum Free DMEM, followed by addition of 11 ml Serum Free DMEM. 2.0 ml of the transfection mixture was then added to the cells, followed by incubation for 5hrs at 37°C/5% CO_2. On day 3, cells were trypsinized and counted, followed by plating of $1x10^6$ cells/well

(12-well plates). Cells were permitted to adhere to the wells for 8 hrs., followed by one wash with 1x PBS. Thereafter, 0.5 μCi ^{3}H-inositol in 1 ml inositol-free DMEM was added per well.

On day 4, the cells were washed with 1.5 ml PBS and then 0.9 ml of assay medium was added containing inositol-free/serum free media, 10 μM pargyline, 10 mM lithium chloride, for 5 min in 37°C/5% CO_2 followed by 100 μl addition of candidate compound diluted in the same material. The cells were then incubated for 120 minutes at 37°C. Then the cells were washed with 1.5 ml PBS and 200 μl of fresh/icecold stop solution (1M KOH; 18 mM Na-borate; 3.8 mM EDTA) was added/well. The solution was kept on ice for 5-10 minutes or until the cells were lysed and then neutralized by 200 μl of fresh/ice cold neutralization sol. (7.5 % HCL). The lysate was then transferred into 1.5 ml micro-centrifuge tubes and 1 ml of chloroform/methanol (1:2) was added/tube. The solution was vortexed for 15 seconds and the upper phase was applied to a Biorad AG1-X8 anion exchange resin (100-200 mesh). The resin was washed with water and 0.9 ml of the upper phase was loaded onto the column. The column was washed with 10 mls of 5 mM myo-inositol and 10 ml of 5 mM Na-borate/60mM Na-formate. The inositol trisphosphates were eluted into scintillation vials containing 10 ml of scintillation cocktail with 2 ml of 0.1 M formic acid/ 1 M ammonium formate. The columns were regenerated by washing with 10 ml of 0.1 M formic acid/3M ammonium formate and rinsed twice with dd H_2O and stored at room temperature in water.

Following this round of assaying, candidate compounds having an IC_{50} value of less than 10μM were considered as potential leads for the development of pharmaceutical compositions.

SCREENING CANDIDATE COMPOUNDS

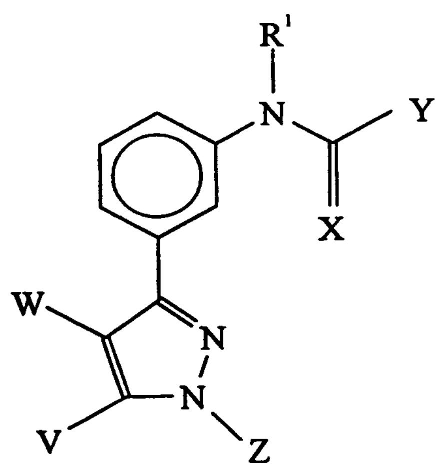
Following the protocols set forth above, one compound, 103487 (Example 8, *supra*) evidenced the following results:

Figure Number	GTPγS AP-1 Percent Inhibition Relative	GTPγS AP-1 Percent Inhibition Relative To	Competitive Binding AP-1 ([^{3}H]mesulergine)	Competitive Binding WT 5HT2A ([^{3}H]LSD)	Inositol Phosphate Accumulation AP-3

	To Positive Control (Primary)	Positive Control (Reconfirm)	IC_{50} Value (nM)	IC_{50} Value (nM)	IC_{50} Value (nM)
15A (103487)	-1%	31%	2100 850	46	52 90

Based upon these results, structure activity analysis of the 103487 compound suggested that a series of derivatives of 3-(4-bromo-1-methylpyrazole-3-yl)phenylamine would exhibit similar 5-HT_{2A} activity and selectivity. A series of derivatives of 3-(4-bromo-1-methylpyrazole-3-yl)phenylamine have now been synthesized. These "directed" library compounds (Tripos, Inc.) were then analyzed in accordance with the protocols of Examples 9c(1), 9c(2) and 9d.

This series of compounds exhibits highly selective 5-HT_{2A} activity. Accordingly, in the first aspect of the invention, a series of compounds possessing 5-HT_{2A} receptor activity that are useful as inverse agonists at such receptors is designated by the general formula (A):



(A)

Wherein:

W is lower alkyl (C_{1-6}), or halogen;

V is lower alkyl (C_{1-6}), or halogen;

X is either Oxygen or Sulfur;

Y is NR^2R^3, or $(CH_2)_mR^4$, or $O(CH_2)_nR^4$;

Z is lower alkyl (C_{1-6});

m = 0 - 4

n = 0 - 4

R^1 is H or lower alkyl (C_{1-4});

R^2 is H or lower alkyl(C_{1-4});

R^3 and R^4 are independently a C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, OCF_3, SMe, $COOR^7$, $SO_2NR^5R^6$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, and aryloxy wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, or aryloxy groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, $NHCOCH_3$, OCF3, SMe, $COOR^7$, SO_3R^7, $SO_2NR^5R^6$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl;

R^5 and R^6 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2 aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^7R^8$, NR^7R^8, $NHCOCH_3$, OCF_3, SMe, $COOR^9$, SO_3R^7, $SO_2NR^7R^8$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, or aryl groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^8R^9$, NR^8R^9, $NHCOCH_3$, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl,

or R^5 and R^6 may form part of a 5, 6 or 7 membered cyclic structure which may be either saturated or unsaturated and that may contain up to four heteroatoms selected from O, N or S and said cyclic structure may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me,

NO_2, OH, OMe, OEt, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$, SO_3R^7, $NHCOCH_3$, COEt, COMe, or halogen;

R^7 may be independently selected from H or C_{1-6} alkyl;

R^8 and R^9 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from halogen, CF_3, OCF3, OEt, CCl_3, Me, NO_2, OH, OMe, SMe, COMe, CN, $COOR^7$, SO_3R^7, COEt, $NHCOCH_3$, or aryl;

an aryl moiety can be a 5 or 6 membered aromatic heterocyclic ring (containing up to 4 hetero atoms independently selected from N, O, or S) or a 6 membered aromatic non-heterocyclic ring or a polycycle;

C_{1-6} alkyl moieties can be straight chain or branched;

optionally substituted C_{1-6} alkyl moieties can be straight chain or branched;

C_{2-6} alkenyl moieties can be straight chain or branched; and

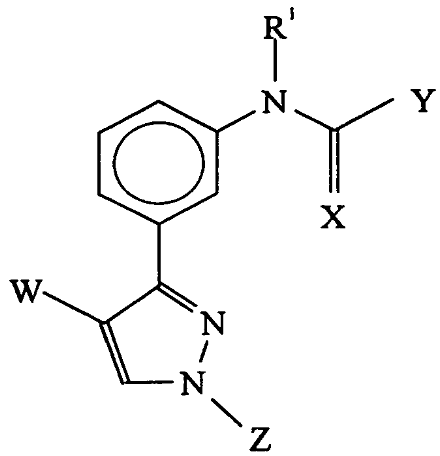
optionally substituted C_{2-6} alkenyl moieties can be straight chain or branched.

Examples of suitable C_{1-6} alkyl groups include but art not limited to methyl, ethyl, n-propyl, i-propyl, n-butyl, and t-butyl.

Halogens are typically F, Cl, Br, and I.

Examples of 5 or 6 membered ring moieties include, but are not restricted to, phenyl, furanyl, thienyl, imidazolyl, pyridyl, pyrrolyl, oxazolyl, isoxazolyl, triazolyl, pyrazolyl, tetrazolyl, thiazolyl and isothiazolyl. Examples of polycycle moieties include, but are not restricted to, naphthyl, benzothiazolyl, benzofuranyl, benzimidazolyl, quinolyl, isoquinolyl, indolyl, quinoxalinyl, quinazolinyl and benzothienyl.

A more preferred series of compounds possessing 5-HT_{2A} receptor activity that are useful as inverse agonists at such receptors is designated by the general formula (B):



(B)

Wherein:

W is Me, or Et, or halogen;

X is either Oxygen or Sulfur;

Y is NR^2R^3, or $(CH_2)_mR^4$, or $O(CH_2)_nR^4$;

Z is lower alkyl (C_{1-6});

m = 0 - 4

n = 0 - 4

R^1 is H or lower alkyl (C_{1-4});

R^2 is H or lower alkyl(C_{1-4});

R^3 and R^4 are independently a C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, OCF_3, SMe, $COOR^7$, $SO_2NR^5R^6$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, and aryloxy wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, or aryloxy groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, $NHCOCH_3$, OCF3, SMe, $COOR^7$, SO_3R^7, $SO_2NR^5R^6$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl;

R^5 and R^6 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2 aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^7R^8$, NR^7R^8, $NHCOCH_3$, OCF_3, SMe, $COOR^9$, SO_3R^7, $SO_2NR^7R^8$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, or aryl groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^8R^9$, NR^8R^9, $NHCOCH_3$, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl,

or R^5 and R^6 may form part of a 5, 6 or 7 membered cyclic structure which may be either saturated or unsaturated and that may contain up to four heteroatoms selected from O, N or S and said cyclic structure may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$, SO_3R^7, $NHCOCH_3$, COEt, COMe, or halogen;

R^7 may be independently selected from H or C_{1-6} alkyl;

R^8 and R^9 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from halogen, CF_3, OCF3, OEt, CCl_3, Me, NO_2, OH, OMe, SMe, COMe, CN, $COOR^7$, SO_3R^7, COEt, $NHCOCH_3$, or aryl;

an aryl moiety can be a 5 or 6 membered aromatic heterocyclic ring (containing up to 4 hetero atoms independently selected from N, O, or S) or a 6 membered aromatic non-heterocyclic ring or a polycycle;

C_{1-6} alkyl moieties can be straight chain or branched;

optionally substituted C_{1-6} alkyl moieties can be straight chain or branched;

C_{2-6} alkenyl moieties can be straight chain or branched; and

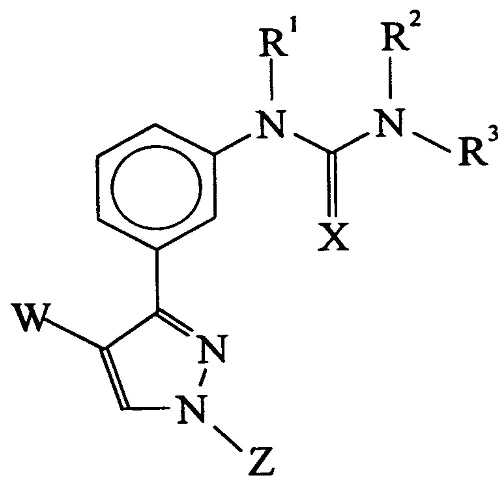
optionally substituted C_{2-6} alkenyl moieties can be straight chain or branched.

Examples of suitable C_{1-6} alkyl groups include but art not limited to methyl, ethyl, n-propyl, i-propyl, n-butyl, and t-butyl.

Halogens are typically F, Cl, Br, and I.

Examples of 5 or 6 membered ring moieties include, but are not restricted to, phenyl, furanyl, thienyl, imidazolyl, pyridyl, pyrrolyl, oxazolyl, isoxazolyl, triazolyl, pyrazolyl, tetrazolyl, thiazolyl and isothiazolyl. Examples of polycycle moieties include, but are not restricted to, naphthyl, benzothiazolyl, benzofuranyl, benzimidazolyl, quinolyl, isoquinolyl, indolyl, quinoxalinyl, quinazolinyl and benzothienyl.

A first series of compounds having 5-HT_{2A} receptor activity is represented by a class (I) of compounds of formula (B) wherein Y=NR^2R^3:



(I)

Wherein:

Preferably R^1 and R^2 are H.

Preferably W is Br.

Preferably X is O.

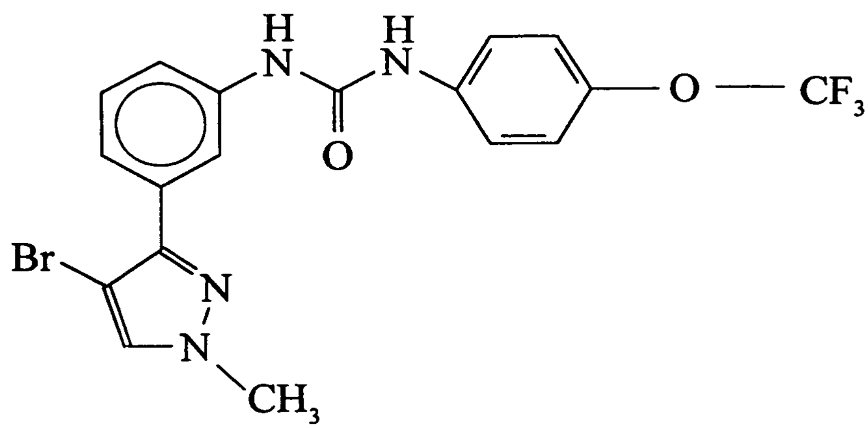
Preferably Z is Me.

Preferably R3 is 4-trifluoromethoxyphenyl or 4-trifluoromethoxybenzyl.

Preferred compounds are:

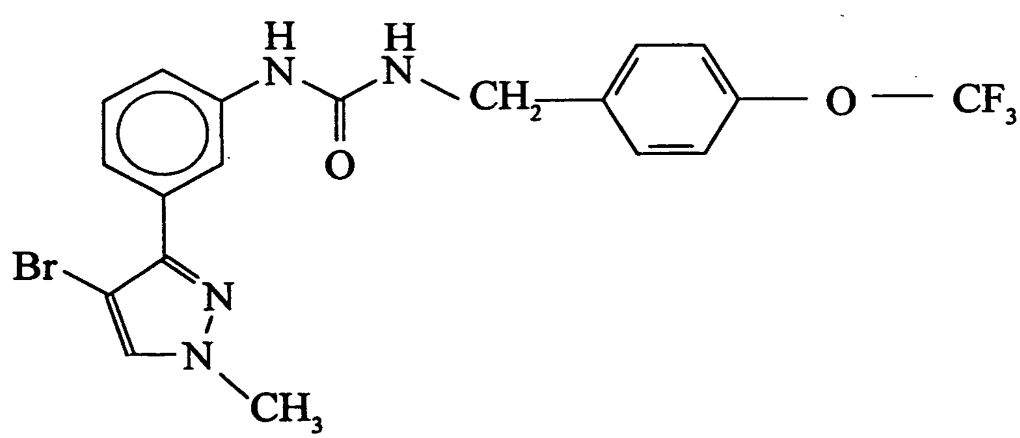
103487

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{(4-trifluoromethoxy)phenyl}amino] carboxamide



116115

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{(4-trifluoromethoxy)phenyl)methyl}amino]carboxamide



These two compounds demonstrated the following activities using the assay protocols defined in the Examples above:

Compound Number	Competitive Binding AP-1 ([^{3}H]mesulergine) IC_{50} Value (μM)	Competitive Binding WT $5HT_{2A}$ ([^{3}H]LSD) IC_{50} Value (μM)	Inositol Phosphate Accumulation AP-3 IC_{50} Value (μM)
103487	2.1	.046	.052
116115	1.2	.45	.0171

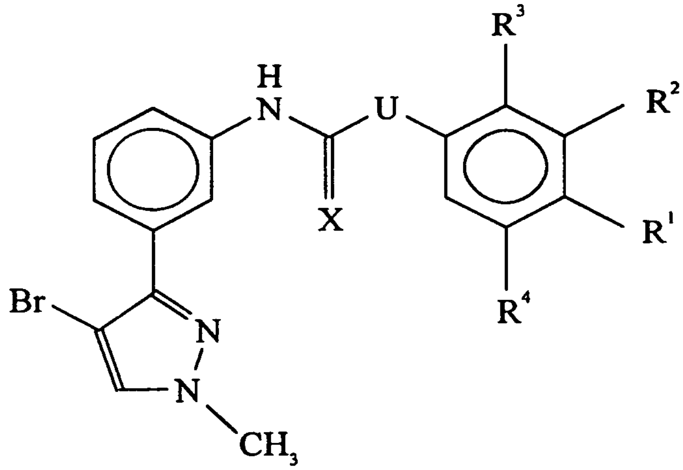
Additional compounds of formula (B) wherein $Y=NR^2R^3$ are set forth below. Inositol phosphate accumulation assays evidence the activity of test compounds. Both single concentration percentages of control values and IC_{50} determinations indicate activity. In the tables below the column legends have the following meanings:

$\underline{IP_3 \text{ \% Contol}}$: The values in this column reflect an IP Accumulation Assay where the test compounds were evaluated at one concentration of 10 μM. For these assays, the compound was diluted into inositol-free Dulbecco's Eagle Media containing 10 μM pargyline and 10 mM LiCl and tested at a final assay concentration of 10 μM, in triplicate. The percent control value was calculated based on the control in which no test compound was added.

$\underline{IP_3 \text{ AP-3 } IC_{50} \text{ nM}}$: The values in this column reflect an IP accumulation assay in which the test compound was evaluated at several different concentrations whereby an IC_{50} could be determined. This column corresponds to the column appearing in the tables above which is labeled: Inositol Phosphate Accumulation, AP-3, IC_{50} Value (μM).

$\underline{\text{WT } 5HT_{2A} \text{ LSD } IC_{50} \text{ nM}}$: The values in this column reflect a competitive binding assay using LSD. This column corresponds to the column appearing in the tables above which is labeled: Competitive Binding, WT $5HT_{2A}$, ([^{3}H]LSD), IC_{50} Value (μM).

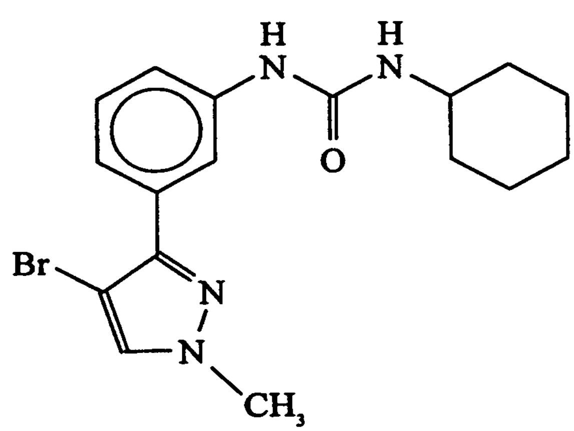
Compounds listed in each of the following tables reference the structures immediately preceding the table. A "dash" in the table indicates that no value was determined.



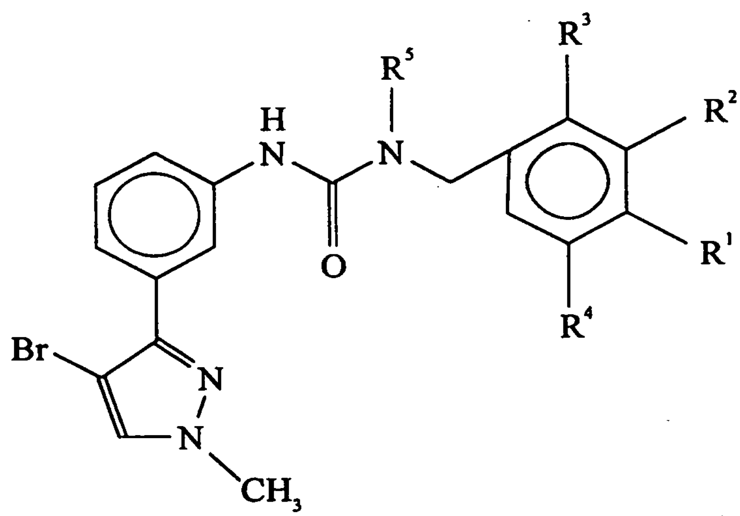
Compound No.	R^1	R^2	R^3	R^4	X	U	IP_3 % of Control	IP_3 AP-3 IC_{50} nM	WT $5HT_{2A}$ LSD IC_{50} nM
N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][(4-methylthiophenyl)amino]carboxamide									
116079	SCH_3	H	H	H	O	NH	16	17	4
N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][(4-chlorophenyl)amino]carboxamide									
116081	Cl	H	H	H	O	NH	10	3.2	11
{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(4-fluorophenyl)carboxamide									
116082	F	H	H	H	O	NH	11	-	7
{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[2-(trifluoromethoxy)phenyl]carboxamide									
116087	H	H	CF_3O	H	O	NH	11	-	200
{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-nitrophenyl)carboxamide									
116089	H	H	NO_2	H	O	NH	27	-	238

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(4-methoxyphenyl)carboxamide									
116091	MeO	H	H	H	O	NH	12	-	19
{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-methylphenyl)carboxamide									
116092	H	H	Me	H	O	NH	32	-	131
{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[4-(trifluoromethyl)phenyl]carboxamide									
116097	CF_3	H	H	H	O	NH	11	-	65
{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(3-chlorophenyl)carboxamide									
116105	H	Cl	H	H	O	NH	11	-	39
{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-chlorophenyl)carboxamide									
116108	H	H	Cl	H	O	NH	6	-	249
{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[4-(methylethyl)phenyl]carboxamide									
116110	isopropyl	H	H	H	O	NH	7	-	338
{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(3-methoxyphenyl)carboxamide									
116111	H	MeO	H	H	O	NH	7	-	106
{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(3-methylphenyl)carboxamide									
116112	H	Me	H	H	O	NH	14	-	57
[{3-(4-bromo-1-methylpyrazol-3-yl)phenyl}amino]-N-methyl-N-[4-(trifluoromethoxy)phenyl]carboxamide									
116113	CF_3O	H	H	H	O	NCH_3	-	193	2
N-[4-(tert-butyl)phenyl]{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}carboxamide									
116119	t-butyl	H	H	H	O	NH	17	-	476
N-[4-(dimethylamino)phenyl]{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}carboxamide									
116122	NMe_2	H	H	H	O	NH	9	-	309

N-(3,5-dichloro-4-methylphenyl){[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}carboxamide									
116138	Me	Cl	H	Cl	O	NH	23	-	122
{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[4-(trifluoromethylthio)phenyl]carboxamide									
116139	CF_3S	H	H	H	O	NH	12	-	56
{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-fluorophenyl)carboxamide									
116144	H	H	F	H	O	NH	12	-	37
2-({[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}carbonylamino)benzamide									
116145	H	H	$CONH_2$	H	O	NH	31	-	7473
{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(4-cyanophenyl)carboxamide									
116147	CN	H	H	H	O	NH	12	-	2
{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-cyanophenyl)carboxamide									
116148	H	H	CN	H	O	NH	30	-	348

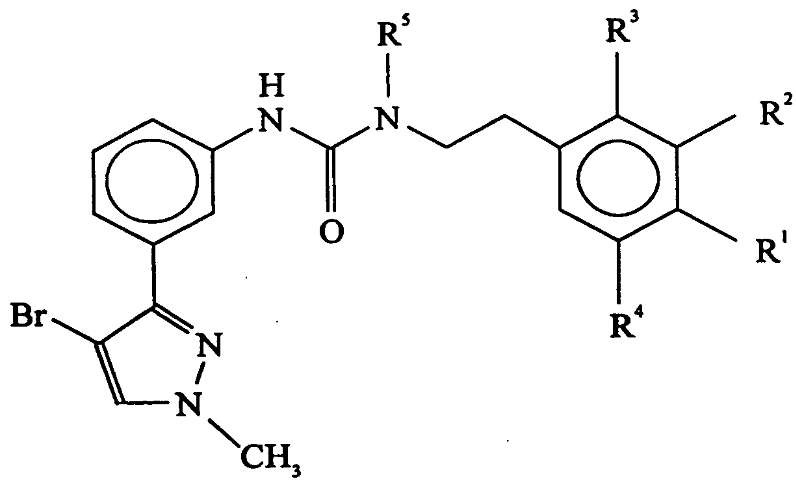


Compound No.	N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][cyclohexylamino]carboxamide	IP3 AP-3 IC50 nM	WT 5HT2A LSD IC50 nM
116141		114	81



Compound No.	R^1	R^2	R^3	R^4	R^5	IP3 AP-3 IC50 nM	WT 5HT2A LSD IC50 nM
N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][phenylmethylamino]carboxamide							
116143	H	H	H	H	H	120	47
N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{(4-fluorophenyl)methyl}amino]carboxamide							
116182	F	H	H	H	H	89	132

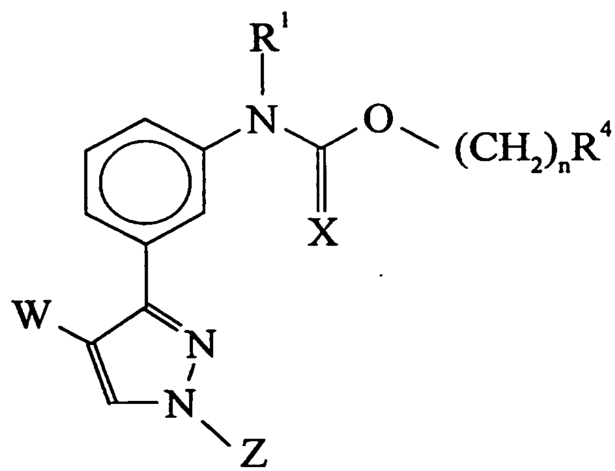
N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{(3,4-dimethoxyphenyl)methyl}amino]carboxamide							
116183	OMe	OMe	H	H	H	-	1010
N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{(3,4,5-trimethoxyphenyl)methyl}amino]carboxamide							
116184	OMe	OMe	H	OMe	H	-	2960
N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{(2-methylphenyl)methyl}amino]carboxamide							
116185	H	H	Me	H	H	-	769
N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{(4-methoxyphenyl)methyl}amino]carboxamide							
116189	OMe	H	H	H	H	-	102



Compound No.	R^1	R^2	R^3	R^4	R^5	**IP_3 AP-3 IC_{50} nM**	**WT $5HT_{2A}$ LSD IC_{50} nM**
N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{2-(4-methoxyphenyl)ethyl}amino]carboxamide							

116194	OMe	H	H	H	H	32	61

A second series of compounds having 5-HT_{2A} receptor activity is represented by a class (II) of compounds of formula (B) wherein Y= $O(CH_2)_nR4$:



(II)

Wherein:

Preferably R^1 is H.

Preferably W is Br.

Preferably X is O.

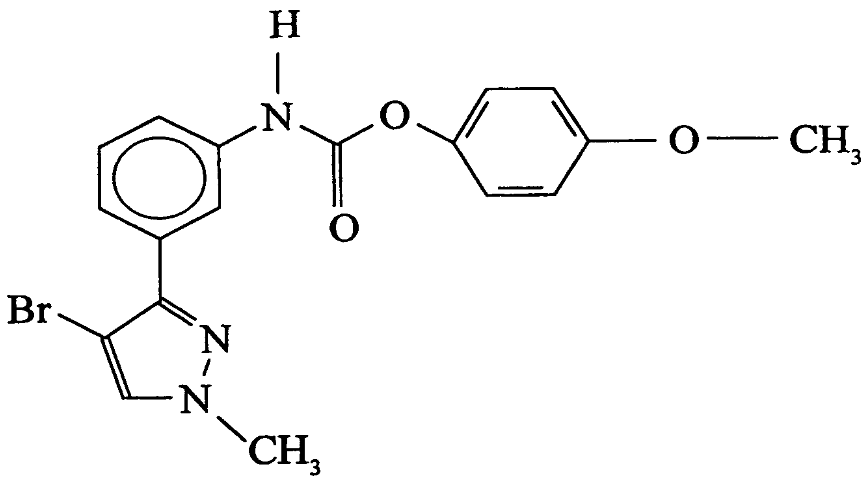
Preferably Z is Me.

Preferably when n = 0, R^4 is 4-methoxyphenyl or tertiary butyl.

Preferred compounds are:

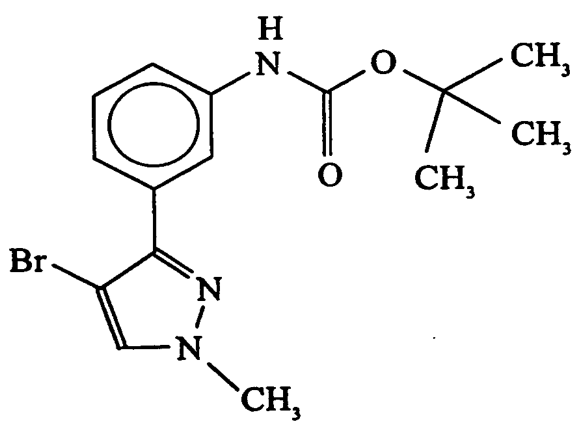
116100

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][4-methoxyphenoxy]carboxamide



116192

(tert-butoxy)-N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]carboxamide



These two compounds demonstrated the following activity:

Compound No.	Competitive Binding AP-1 ([^{3}H]mesulergine) IC_{50} Value (μM)	Competitive Binding WT $5HT_{2A}$ ([^{3}H]LSD) IC_{50} Value (μM)	Inositol Phosphate Accumulation AP-3 IC_{50} Value (μM)
116100	1.8	<0.001	0.0003

116192	-	0.014	0.057

In addition to the assays discussed above, the specific activity of 116100 at the $5HT_{2A}$ receptor was further confirmed by the following.

In Vitro Binding of $5HT_{2A}$ Receptor

Animals:

Animals (Sprague-Dawley rats) were sacrificed and brains were rapidly dissected and frozen in isopentane maintained at -42°C. Horizontal sections were prepared on a cryostat and maintained at -20°C.

LSD Displacement Protocol:

Lysergic acid diethylamide (LSD) is a potent 5HT2A receptor and dopamine D2 receptor ligand. An indication of the selectivity of compounds for either or both of these receptors involves displacement of radiolabeled-bound LSD from pre-treated brain sections. For these studies, radiolabeled I^{125}-LSD (NEN Life Sciences, Boston, MA., Catalogue number NEX-199) was utilized; spiperone (RBI, Natick, MA. Catalogue number s-128), a 5HT2A receptor and dopamine D2 receptor antagonist, was also utilized. Buffer consisted of 50 nanomolar TRIS-HCl, pH 7.4

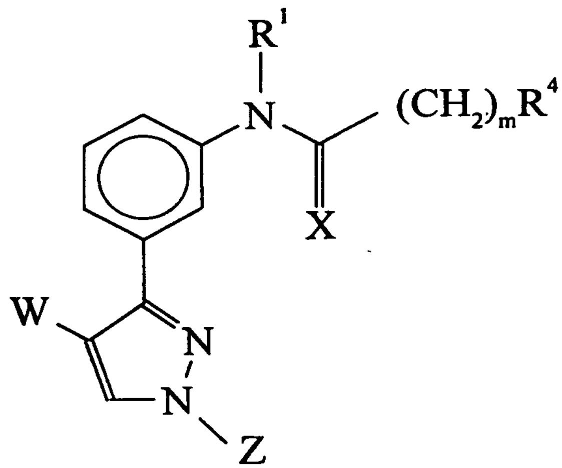
Brain sections were incubated in (a) Buffer plus 1 nanomolar I^{125}-LSD; (b) Buffer plus 1 nanomolar I^{125}-LSD and 1 micromolar spiperone; or Buffer plus 1 nanomolar I^{125}-LSD and 1 micromolar 116100 for 30 minutes at room temperature. Sections were then washed 2X 10 minutes at 4°C in Buffer, followed by 20 seconds in distilled H_2O. Slides were then air-dried.

After drying, sections were apposed to x-ray film (Kodak Hyperfilm) and exposed for 4 days.

Analysis:

Figures 16A-C provide representative autoradiographic sections from this study. Figure 16A evidences darker bands (derived from I^{125}-LSD binding) primarily in both the fourth layer of the cerebral cortex (primarily $5HT_{2A}$ receptors), and the caudate nucleus (primarily dopamine D2 receptors and some $5HT_{2A}$ receptors). As can be seen from Figure 16B, spiperone, which is a $5HT_{2A}$ and dopamine D2 antagonist, displaces the I^{125}-LSD from these receptors on both the cortex and the caudate. As can be further seen from Figure 16C, 116100 appears to selectively displace the I^{125}-LSD from the cortex ($5HT_{2A}$) and not the caudate (dopamine D2).

A third series of compounds having 5-HT_{2A} receptor activity is represented by a class (III) of compounds of formula (B) wherein Y= $(CH_2)_mR^4$:



(III)

Wherein:

Preferably W is Br.

Preferably X is O.

Preferably Z is Me.

Preferably R^1 is H.

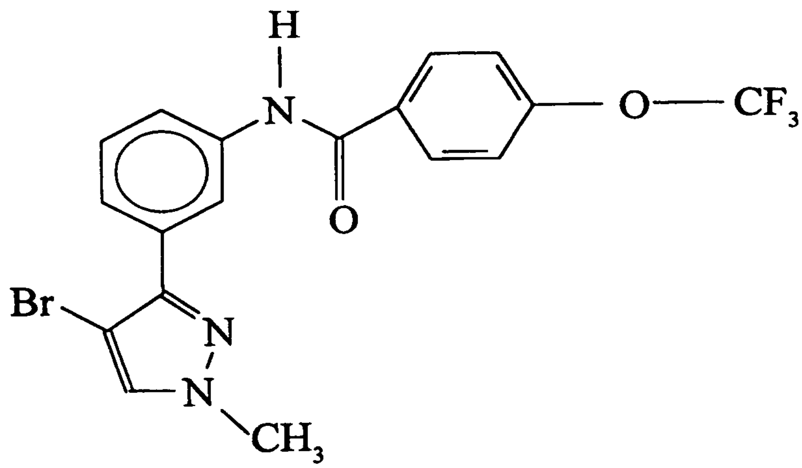
Preferably when m = 0, R^4 is preferably 4-trifluoromethoxyphenyl, or thiophene, or 4-chlorophenyl.

Preferred compounds are:

116101

m = 0, R^1 = H, R^4 = 4-trifluoromethoxyphenyl

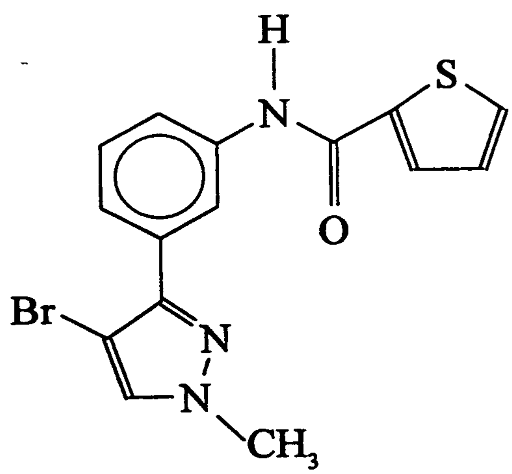
N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][4-trifluoromethoxyphenyl]carboxamide



116102

$m = 0$, $R^1 = H$, R^4 = thiophene

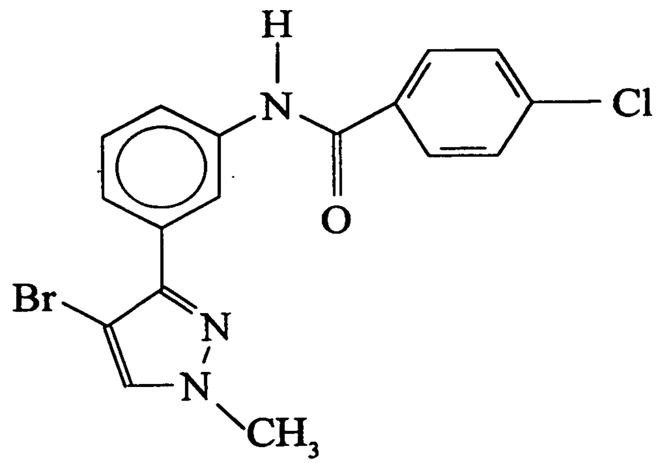
N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][2-thienyl]carboxamide



116120

$m = 0$, $R^1 = H$, R^4 = 4-chlorophenyl

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][4-chl rophenyl]carboxamide



These three compounds demonstrated the following activities:

Compound Number	Competitive Binding AP-1 ([^{3}H]mesulergine) IC_{50} Value (μM)	Competitive Binding WT $5HT_{2A}$ ([^{3}H]LSD) IC_{50} Value (μM)	Inositol Phosphate Accumulation AP-3 IC_{50} Value (μM)
116101	6.1	.46	0.0213
116102	2.8	.17	0.080
116120	1.2	.21	0.0315

In Vivo Analysis of Compound 116102

In addition to the in vitro assays shown in the above table, the in vivo response of animals to the 116102 compound is demonstrated by the following.

A $5HT_{2A}$ receptor antagonist or inverse agonist is expected to decrease amphetamine-stimulated locomotion without affecting baseline locomotion. *See, for example*, Soresnon, et al, 266(2) J. Pharmacol. Exp. Ther. 684 (1993). Based upon the foregoing information, Compound 116102 is a potent inverse agonist at the human 5HT2A receptor. For the following study, the following parameters and protocol were utilized:

Animals, Vehicle

Adult male Sprague-Dawley rats were utilized for these studies. Animals were housed in groups of 2-3 in hanging plastic cages with food and water available at all times. Animals were weighed and handled for at least one day prior to surgery and throughout the studies. For these studies, Vehicle consisted of 90% ethanol (100%) and 10% water.

Amphetamine-stimulated locomotor activity: Assessment and Apparatus

A San Diego Instruments Flex Field apparatus was used to quantify baseline and amphetamine-stimulated locomotor activity. This apparatus consists of four 16" x 16" clear plastic open fields. Photocell arrays (16 in each dimension) interfaced with a personal computer to automatically quantify activity. Several measures of activity can be assessed with the apparatus, including total photocell beam breaks. Animals (vehicle control and Compound treated) were injected s.c. 30 minutes prior to initiation of analysis. Following this 30 minute period, animals were placed individually into an open field and baseline activity was assessed for 30 minutes (habituation phase). Following baseline, animals were removed, injected with d-amphetamine sulfate (1.0 mg/kg) and immediately returned to the open field for 150 minutes, in order to follow the time course (10 minute intervals) of amphetamine-stimulated locomotor activity.

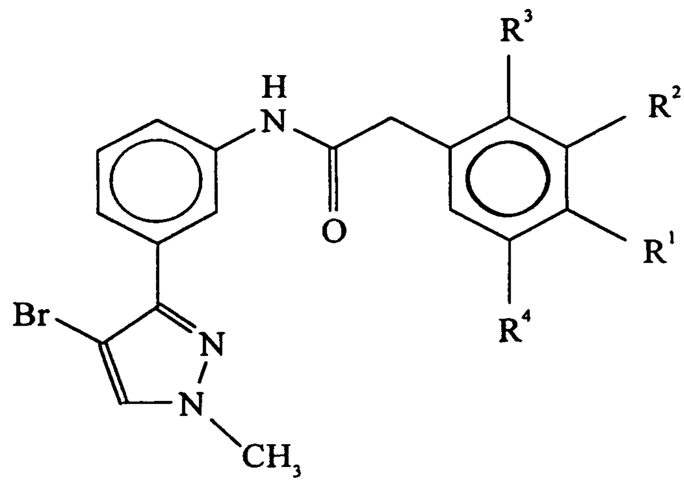
Dosing

Vehicle Control	**Compound 116102**	**Dose (mg/kg)**
6 animals	6 animals	0.1
	6 animals	1.0
	6 animals	5.0
	6 animals	10.0

Analysis

Results, based upon the number of recorded photobeam breaks (mean +/- sem), are presented in Figure 17A-C. As supported by Figures 17A,B and C, a general "inverted U" shaped pattern was observed (*see, generally*, Sahgal, A. "Practical behavioural neuroscience: problems, pitfalls and suggestions" pp 1-8, 5 in Behavioral Neuroscience: A Practical Approach, Volume 1 A. Sahgal (Ed.) 1993, IRL Press, New York). As Figure 17 also indicates, with exception of the highest dose (10mg/kg), *in vivo*, the tested doses of Compound 116102 evidenced a decrease in the amphetamine-stimulated locomotion, consistent with a 5HT2A receptor antagonist or inverse agonist.

Additional compounds of formula (B) wherein Y= $(CH_2)_mR^4$ are set forth below.

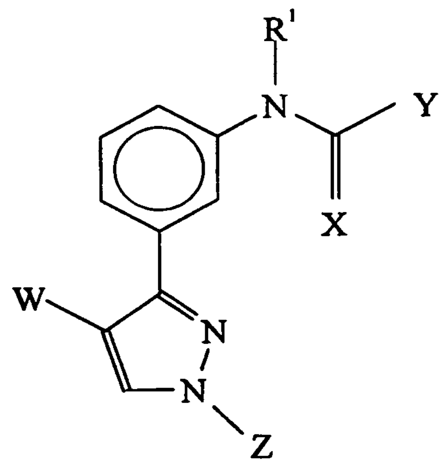


Compound No.	R^1	R^2	R^3	R^4	IP_3 IC_{50} nM	LSD IC_{50} nM
N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]-2-[4-(trifluoromethoxy)phenyl]acetamide						
116137	OCF_3	H	H	H	-	106
N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]-2-(3-fluorophenyl)acetamide						
116174	H	F	H	H	153	318
N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]-2-(3-methoxyphenyl)acetamide						
116175	H	OMe	H	H	108	625
N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]-2-(2-fluorophenyl)acetamide						
116176	H	H	F	H	129	662
N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]-2-(4-nitrophenyl)acetamide						
116177	NO_2	H	H	H	61	108

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]-2-(2-methoxyphenyl)acetamide						
116178	H	H	OMe	H	165	2300

compound names not provided

Based upon the discovery of the specific inverse agonist activity of the above identified compounds at the $5HT_{2A}$ receptor, a novel class of compounds has been identified which exhibits said activity. Accordingly, in the second aspect of the invention, there is provided a novel compound of formula (C):



(C)

Wherein:

W is Me, or Et, or halogen;

X is either Oxygen or Sulfur;

Y is NR^2R^3, or $(CH_2)_mR^4$, or $O(CH_2)_nR^4$;

Z is lower alkyl (C_{1-6});

m = 0 - 4;

n = 0 - 4;

R^1 is H or lower alkyl *(C_{1-4})*;

R^2 is H or lower alkyl(C_{1-4});

R^3 is a C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or $(CH_2)_k$aryl group (k = 1 – 4), preferably k = 1, and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH,

OMe, OEt, $CONR^5R^6$, NR^5R^6, OCF_3, SMe, $COOR^7$, $SO_2NR^5R^6$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, and aryloxy wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, or aryloxy groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, $NHCOCH_3$, OCF3, SMe, $COOR^7$, SO_3R^7, $SO_2NR^5R^6$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl;

R^4 is a C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, OCF_3, SMe, $COOR^7$, $SO_2NR^5R^6$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, and aryloxy wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, or aryloxy groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, $NHCOCH_3$, OCF3, SMe, $COOR^7$, SO_3R^7, $SO_2NR^5R^6$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl;

R^5 and R^6 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2 aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^7R^8$, NR^7R^8, $NHCOCH_3$, OCF_3, SMe, $COOR^9$, SO_3R^7, $SO_2NR^7R^8$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, or aryl groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^8R^9$, NR^8R^9, $NHCOCH_3$, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl,

or R^5 and R^6 may form part of a 5, 6 or 7 membered cyclic structure which may be either saturated or unsaturated and that may contain up to four heteroatoms selected from O, N or S and said cyclic structure may be optionally substituted by up

to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$, SO_3R^7, $NHCOCH_3$, COEt, COMe, or halogen;

R^7 may be independently selected from H or C_{1-6} alkyl;

R^8 and R^9 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from halogen, CF_3, OCF3, OEt, CCl_3, Me, NO_2, OH, OMe, SMe, COMe, CN, $COOR^7$, SO_3R^7, COEt, $NHCOCH_3$, or aryl;

an aryl moiety can be a 5 or 6 membered aromatic heterocyclic ring (containing up to 4 hetero atoms independently selected from N, O, or S) or a 6 membered aromatic non-heterocyclic ring or a polycycle;

C_{1-6} alkyl moieties can be straight chain or branched;

optionally substituted C_{1-6} alkyl moieties can be straight chain or branched;

C_{2-6} alkenyl moieties can be straight chain or branched; and

optionally substituted C_{2-6} alkenyl moieties can be straight chain or branched;

with the proviso that said compound is not:

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][methylamino]carboxamide, or

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{(4-trifluoromethoxy)phenyl}amino] carboxamide, or

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][2-chlorophenyl]carboxamide, or

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][2-chloro-3-pyridyl]carboxamide, or

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][trichloromethyl]carboxamide.

Examples of suitable C_{1-6} alkyl groups include but art not limited to methyl, ethyl, n-propyl, i-propyl, n-butyl, and t-butyl.

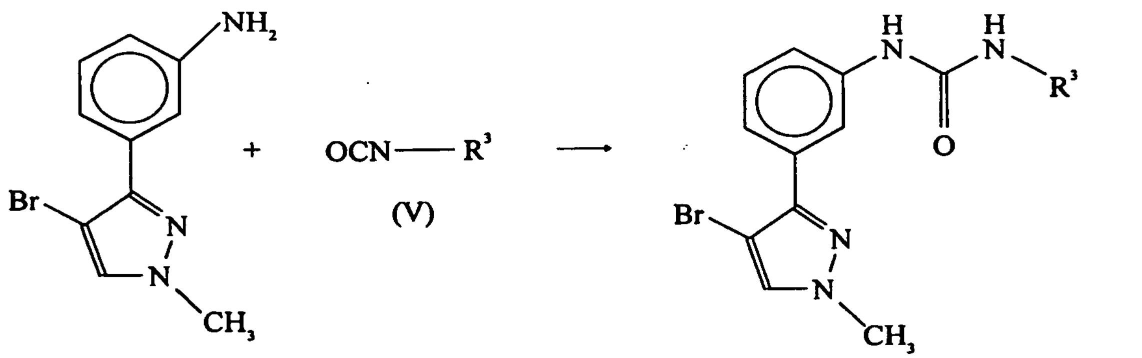
Halogens are typically F, Cl, Br, and I.

Examples of 5 or 6 membered ring moieties include, but are not restricted to, phenyl, furanyl, thienyl, imidazolyl, pyridyl, pyrrolyl, oxazolyl, isoxazolyl, triazolyl, pyrazolyl, tetrazolyl, thiazolyl and isothiazolyl. Examples of polycycle moieties include, but are not restricted to, naphthyl, benzothiazolyl, benzofuranyl, benzimidazolyl, quinolyl, isoquinolyl, indolyl, quinoxalinyl, quinazolinyl and benzothienyl.

Synthetic Approaches

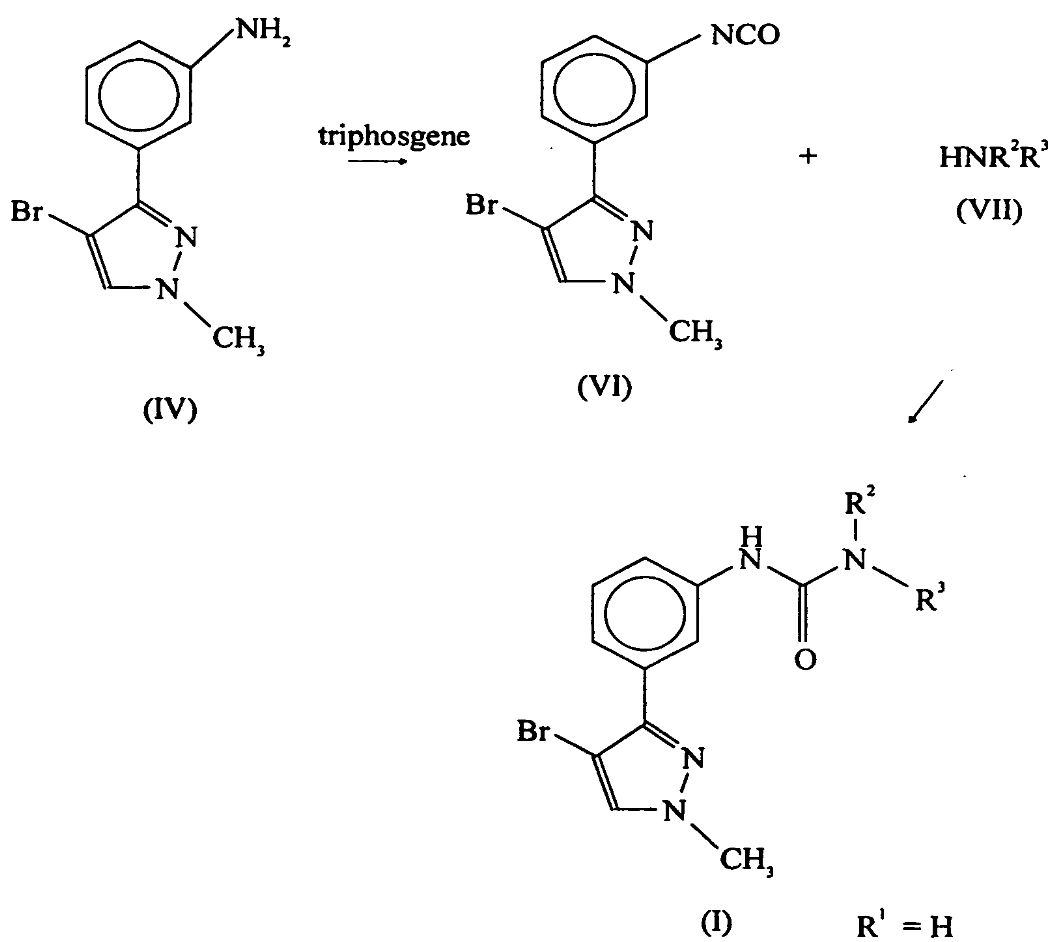
The compounds disclosed in this invention may be readily prepared according to a variety of synthetic manipulations, all of which would be familiar to one skilled in the art. In the general syntheses set forth below, the labeled substituents have the same identifications as set out in the definitions of the compounds above.

Compounds of general formula (I) can be obtained *via* a variety of synthetic routes all of which would be familiar to one skilled in the art. The reaction of isocyanates with amines is a commonly practised method for the formation of ureas (see Org. Syn. Coll. Vol. V, (1973), 555). Amine (IV), 3-(4-bromo-1-methylpyrazole-3-yl)phenylamine, commercially available from Maybridge Chemical Company, Catalog No. KM01978, CAS No. 175201-77-1] reacts readily with isocyanates (V) in inert solvents such as halocarbons to yield the desired ureas of general formula (I) wherein $R^1 = R^2 = H$:

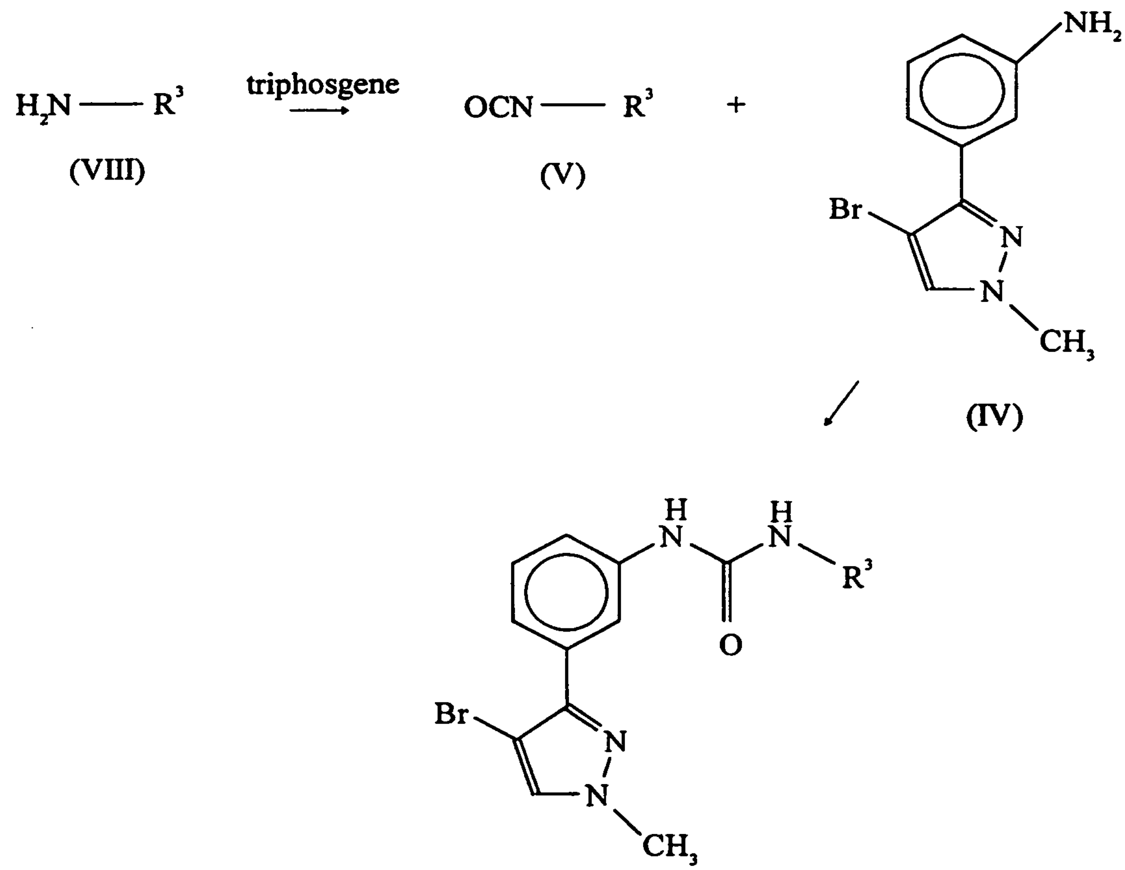


(IV) (I) $R^1 = R^2 = H$

Alternatively the amine (IV) can be converted to the corresponding isocyanate (VI) by the action of phosgene or a suitable phosgene equivalent, e.g. triphosgene, in an inert solvent such as a halocarbon in the presence of an organic base such as triethylamine or ethyldiisopropylamine. Isocyanate (VI) reacts with amines of general formula (VII), in an analogous fashion to that described above for the reaction of (IV) with (V), yielding the desired ureas of general formula (I) wherein $R^1 = H$:

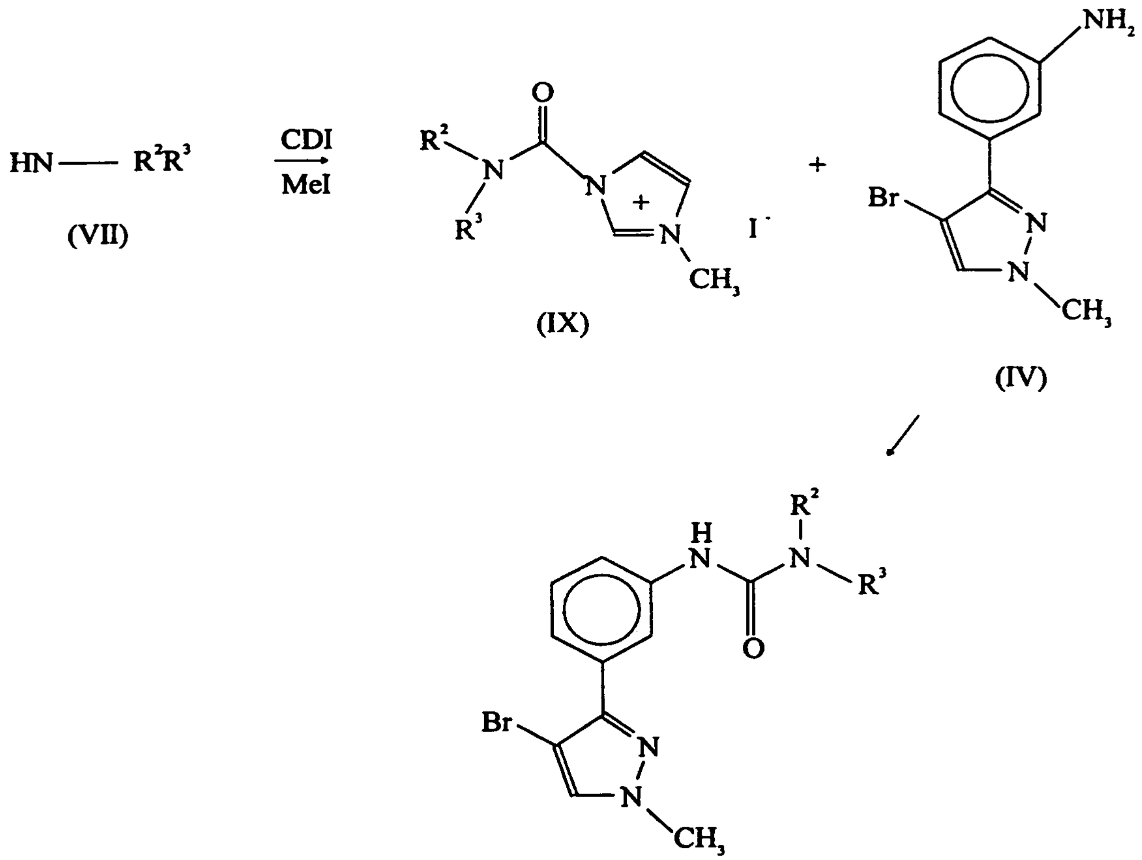


Alternatively wherein the isocyanate of general formula (V) is not commercially available it can be prepared from the corresponding amine of general formula (VIII) in an analogous procedure to that described above for the preparation of (VI). Reaction of these isocyanates with (IV) would again yield the requisite ureas of general formula (I) wherein $R^1 = R^2 = H$:



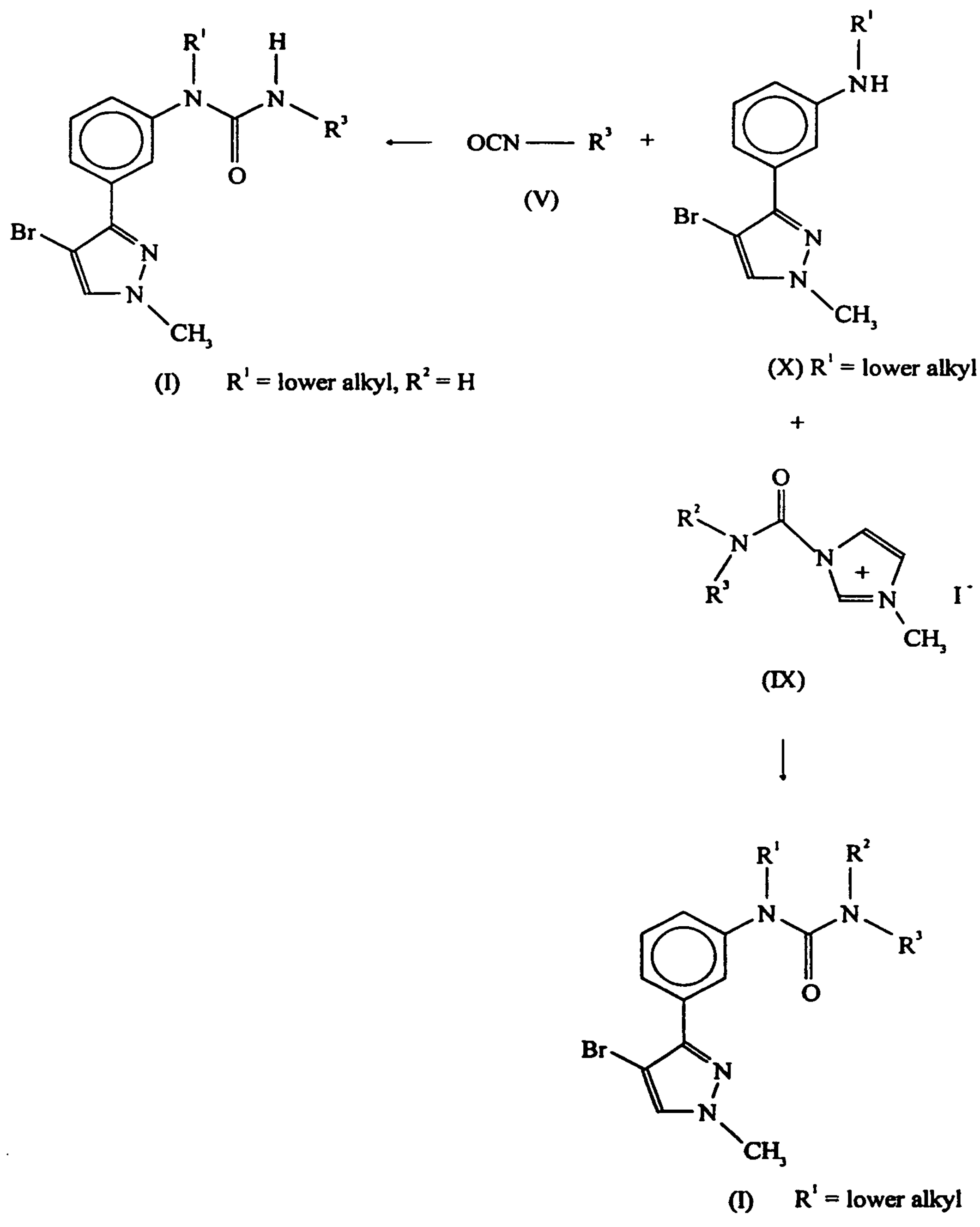
(I) $R^1 = R^2 = H$

Amines of general formula **(VII)** are also readily converted to activated isocyanate equivalents of general formula **(IX)** by the sequential action of carbonyldiimidazole and methyl iodide in tetrahydrofuran and acetonitrile respectively (R.A. Batey *et al*, *Tetrahedron Lett.*, (1998), 39, 6267-6270.) Reaction of **(IX)** with **(IV)** in an inert solvent such as a halocarbon would yield the requisite ureas of general formula **(I)** wherein R^1 = H:



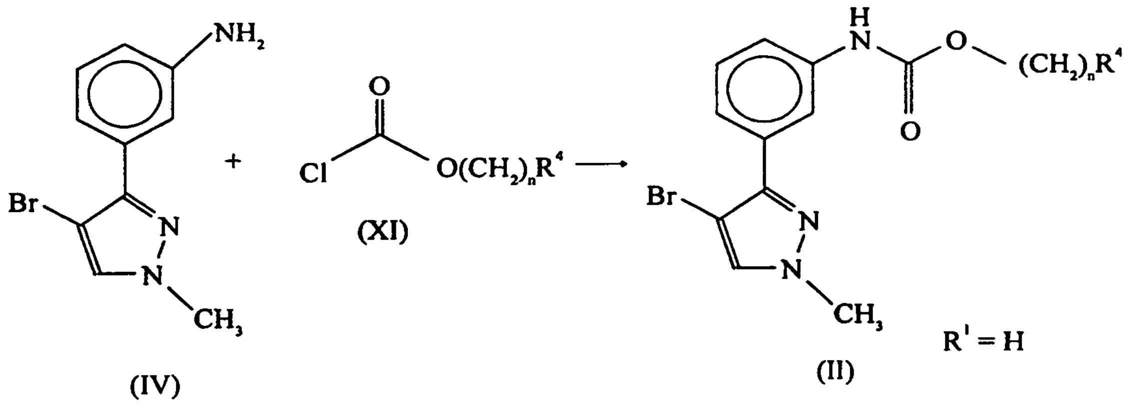
(I) $R^1 = H$

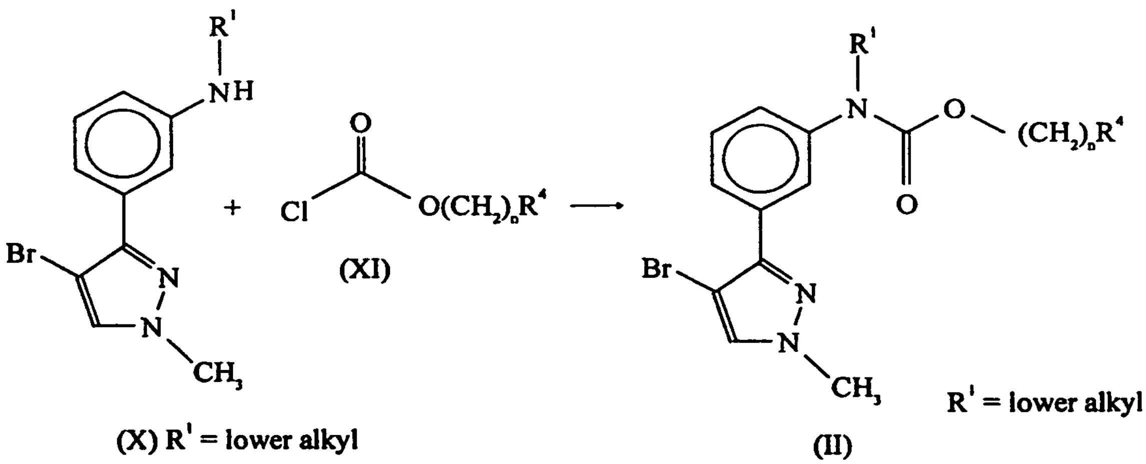
Amine **(IV)** may be monomethylated according to the procedure of J. Barluenga *et al*, *J. Chem. Soc., Chem. Commun.*, (1984), 20, 1334-1335, or alkylated according to the procedure of P. Marchini *et al*, *J. Org. Chem.*, (1975), 40(23), 3453-3456, to yield compounds of general formula **(X)** wherein R^1 = lower alkyl. These materials may be reacted as above with reagents of general formula **(V)** and **(IX)** as depicted below:



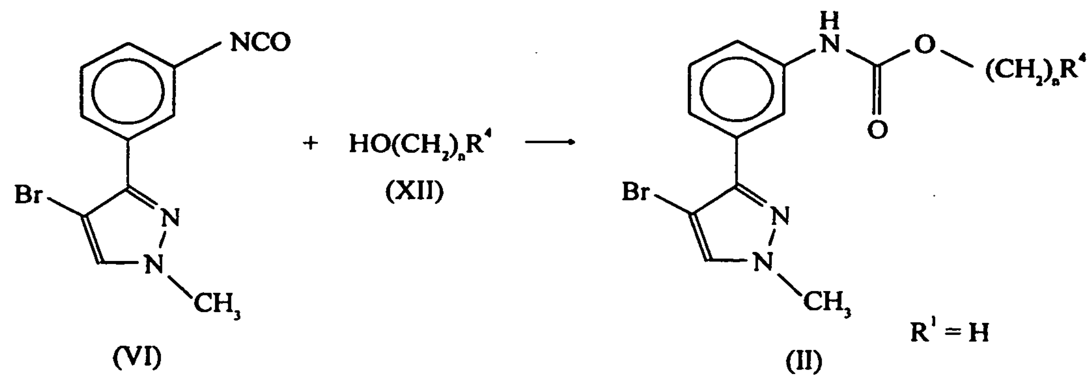
Compounds of general formula (II) can similarly be obtained *via* a variety of synthetic manipulations, all of which would be familiar to one skilled in the art. The reaction of amine (IV) with chloroformates (see Org. Syn. Coll. Vol. IV, (1963), 780) of general formula (XI) in an inert solvent such as ether or halocarbon in the presence of a tertiary base such as triethylamine or ethyldiisopropylamine readily yields the requisite carbamates of general formula (II) wherein R^1 =

H. Analogously amines of general formula **(X)** react similarly with chloroformates **(XI)** to yield the requisite carbamates of general formula **(II)** wherein R^1 = lower alkyl:

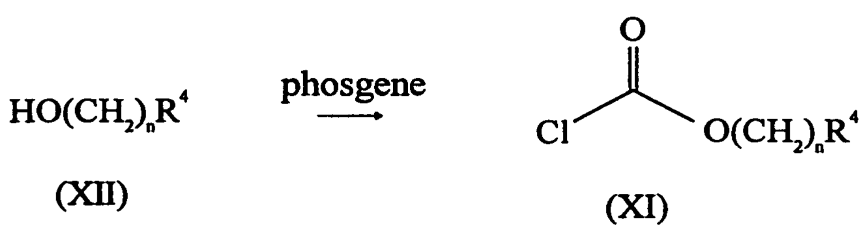




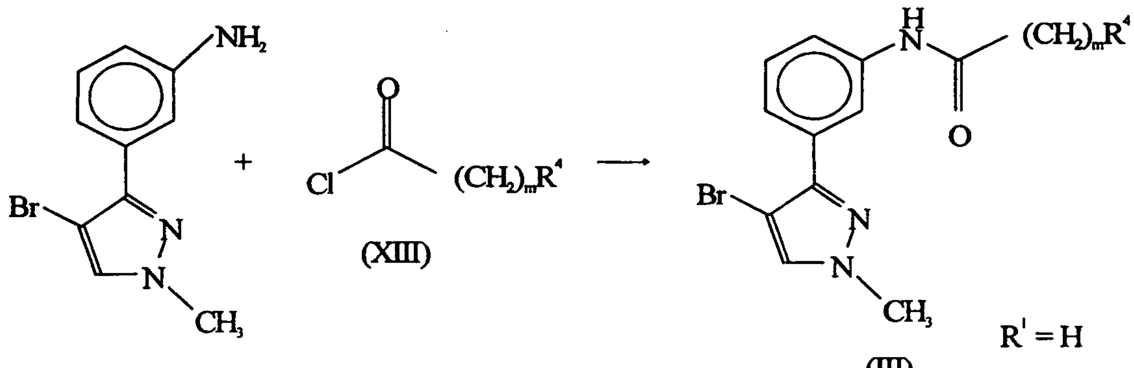
An alternative route employs the ready reaction of an alcohol with an isocyanate. Thus isocyanate (VI) described previously reacts readily with alcohols (XII) in an aprotic solvent such as ether or chlorocarbon to yield the desired carbamates of general formula (II) wherein R^1 = H:



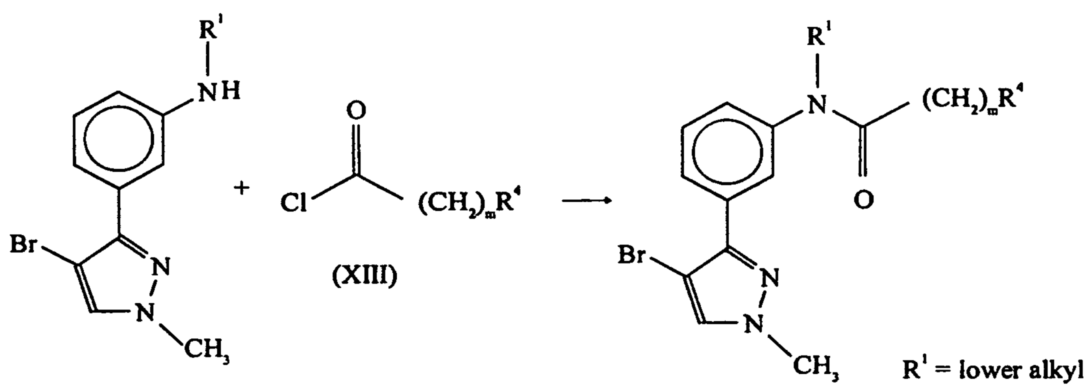
Chloroformates of general formula **(XI)** not commercially available may be readily prepared from the corresponding alcohol **(XII)** in an inert solvent such as toluene, chlorocarbon or ether by the action of excess phosgene (see Org. Syn. Coll. Vol. III, (1955), 167):



Compounds of general formula **(III)** can be obtained *via* a variety of synthetic manipulations, all of which would be familiar to one skilled in the art. The reaction of amine **(IV)** with acid chlorides (see Org. Syn. Coll. Vol. V, (1973), 336) of general formula **(XIII)** to yield the desired amides **(III)** wherein R^1 = H is readily achieved in an inert solvent such as chloroform or dichloromethane in the presence of an organic base such as triethylamine or ethyldiisopropylamine. In an identical fashion amines of general formula **(X)** would react with acid chlorides **(XIII)** to yield the desired amides **(III)** wherein R^1 = lower alkyl:

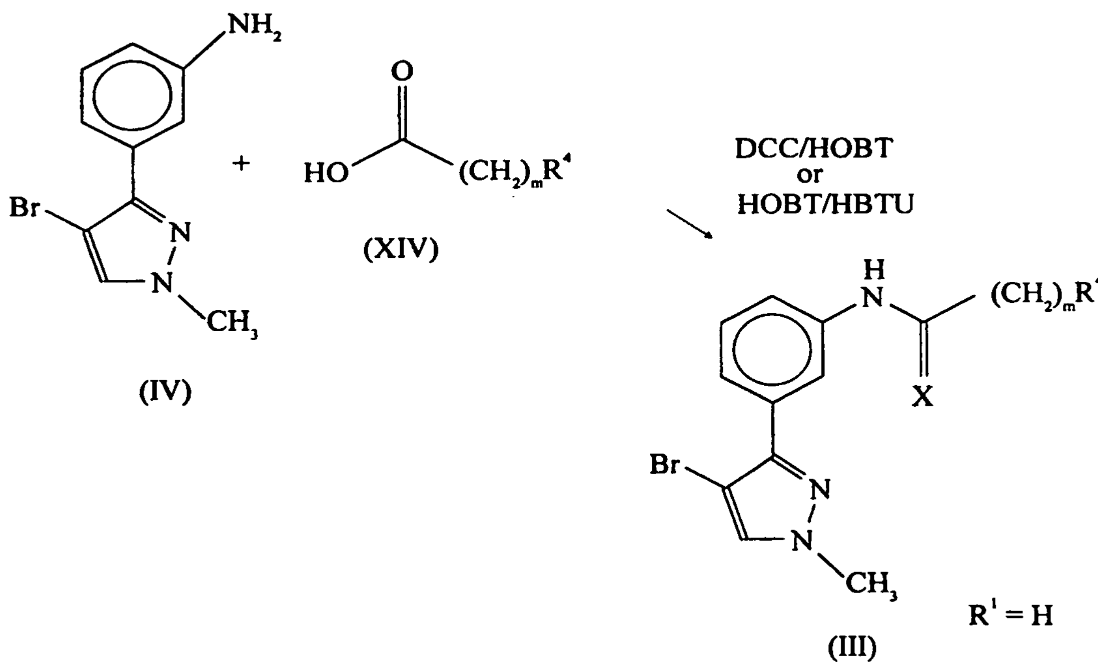


(IV) (XIII) (III) $R^1 = H$

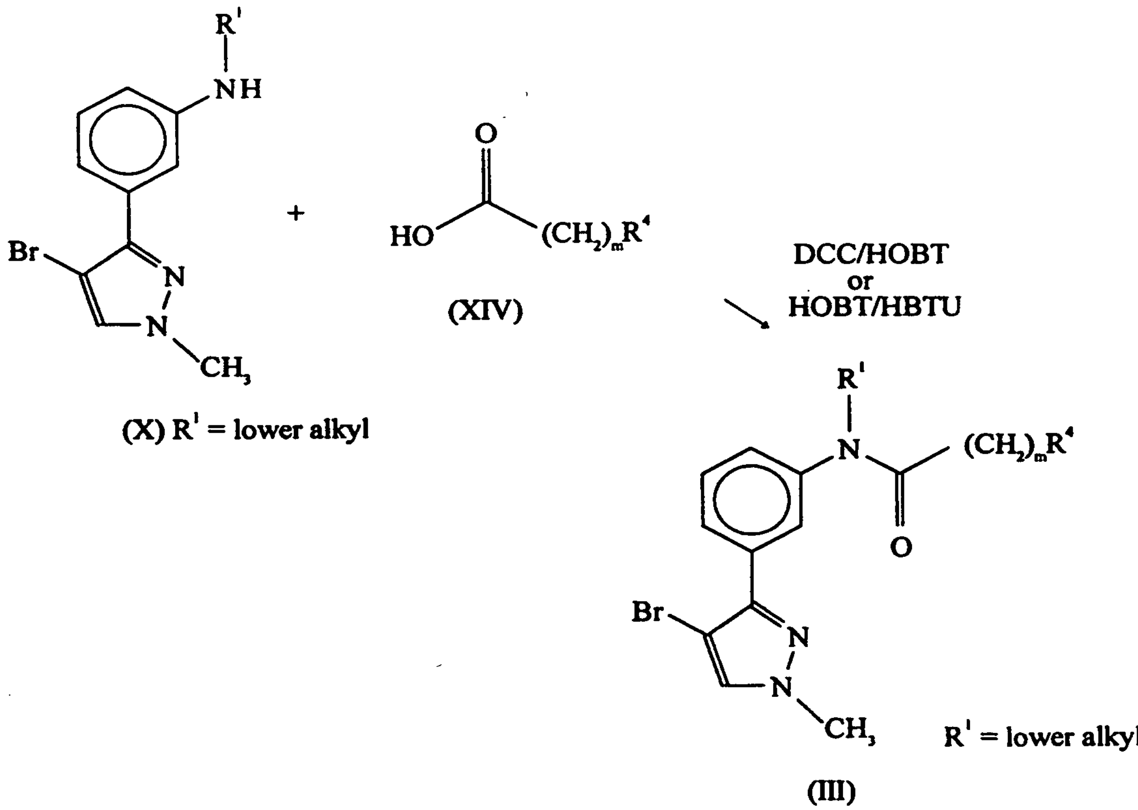


(X) R^1 = lower alkyl (XIII) (III) R^1 = lower alkyl

Alternatively the corresponding acids of general formula **(XIV)** may be coupled with dicyclohexylcarbodiimide (DCC)/hydroxybenzotriazole (HOBT) (see W. Konig *et al*, *Chem. Ber.*, (1970), 103, 788) or hydroxybenzotriazole (HOBT)/2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (see M. Bernatowicz *et al.*, *Tetrahedron Lett.*, (1989), 30, 4645) as condensing agents in dimethylformamide or chloroform to amines **(IV)** and **(X)** respectively yielding products identical to those described in the previous scheme:

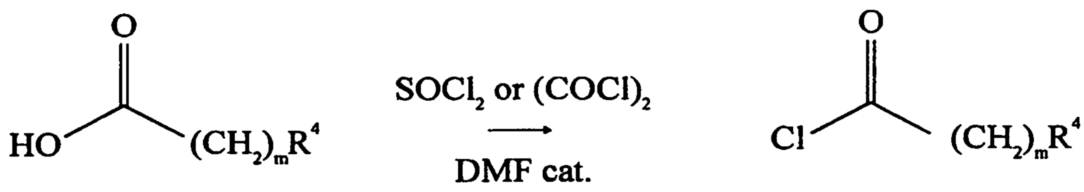


(IV) (XIV) (III) $R^1 = H$



(X) R^1 = lower alkyl (XIV) (III) R^1 = lower alkyl

The acids of general formula **(XIV)** are readily converted to the corresponding acid chlorides **(XIII)** by the action of thionyl chloride or oxalyl chloride in the presence of catalytic dimethylformamide:



(XIV) (XIII)

A third aspect of the present invention provides a compound of formula (A) or a solvate or physiologically functional derivative thereof for use as a therapeutic agent, specifically as a modifier of the activity of the serotonin 5-HT_{2A} receptor. Modifiers of the activity of the serotonin 5-HT_{2A} receptor are believed to be of potential use for the treatment or prophylaxis of CNS, gastrointestinal, cardiovascular, and inflammatory disorders. Compounds of the formula (A) may be administered by oral, sublingual, parenteral, rectal, or topical administration. In addition to the neutral forms of compounds of formula (A) by appropriate addition of an ionizable substituent, which does not alter the receptor specificity of the compound, physiologically acceptable salts of the compounds may also be formed and used as therapeutic agents. Different amounts of the compounds of formula (A) will be required to achieve the desired biological effect. The amount will depend on factors such as the specific compound, the use for which it is intended, the means of administration, and the condition of the treated individual. A typical dose may be expected to fall in the range of 0.001 to 200 mg per kilogram of body weight of the treated individual. Unit does may contain from 1 to 200 mg of the compounds of formula (A) and may be administered one or more times a day, individually or in multiples. In the case of the salt or solvate of a compound of formulas (A), the dose is based on the cation (for salts) or the unsolvated compound.

A fourth aspect of the present invention provides pharmaceutical compositions comprising at least one compound of formula (A) and/or a pharmacologically acceptable salt or solvate thereof as an active ingredient combined with at least one pharmaceutical carrier or excipient. Such pharmaceutical compositions may be used in the treatment of clinical conditions for which a modifier of the activity of the serotonin 5-HT_{2A} receptor is indicated. At least one compound of formula (A) may be combined with the carrier in either solid or liquid form in a unit dose formulation. The pharmaceutical carrier must be compatible with the other ingredients in the composition and must be tolerated by the individual recipient. Other physiologically active ingredients may be incorporated into the pharmaceutical composition of the invention if desired, and if such ingredients are compatible with the other ingredients in the composition. Formulations may be prepared by any suitable method, typically by uniformly mixing the active compound(s) with liquids or finely divided solid carriers, or both, in the required proportions, and then, if necessary, forming the resulting mixture into a desired shape.

Conventional excipients, such as binding agents, fillers, acceptable wetting agents, tabletting lubricants, and disintegrants may be used in tablets and capsules for oral administration. Liquid preparations for oral administration may be in the form of solutions, emulsions, aqueous or oily suspensions, and syrups. Alternatively, the oral preparations may be in the form of dry powder which can be reconstituted with water or another suitable liquid vehicle before use. Additional additives such as suspending or emulsifying agents, non-aqueous vehicles (including edible oils), preservatives, and flavorings and colorants may be added to the liquid preparations. Parenteral dosage forms may be prepared by dissolving the compound of the invention in a suitable liquid vehicle and filter sterilizing the solution before filling and sealing an appropriate vial or ampoule. These are just a few examples of the many appropriate methods well known in the art for preparing dosage forms.

The fifth aspect of the present invention provides for the use of a compound of formula (A) in the preparation of a medicament for the treatment of a medical condition for which a modifier of the activity of the serotonin 5-HT_{2A} receptor is indicated.

A sixth aspect of the present invention provides for a method of treatment of a clinical condition of a mammal, such as a human, for which a modifier of the activity of the serotonin 5-HT_{2A} receptor is indicated, which comprises the administration to the mammal

of a therapeutically effective amount of a compound of formula (A) or a physiologically acceptable salt, solvate, or physiologically functional derivative thereof.

Experimental Data

Mass spectra were recorded on a Micromass Platform LC with Gilson HPLC. Infra-red spectra were recorded on a Nicolet Avatar 360 FT-IR. Melting points were recorded on a Electrothermal IA9200 apparatus and are uncorrected. Proton nuclear magnetic resonance spectra were recorded on a Bruker 300MHz machine. Chemical shifts are given with respect to tetramethylsilane. In the text the following abbreviations are used; s (singlet), d (doublet), t (triplet), m (multiplet) or combinations thereof. Chemical shifts are quoted in parts per million (ppm) and with coupling constants in Hertz.

Thin layer chromatography was carried out using aluminium backed silica plates (250μL; GF_{254}). HPLC was recorded either on a HP Chemstation 1100 HPLC using a Hichrom 3.5 C18 reverse phase column (50mm x 2.1mm i.d.). Linear gradient elution over 5 minutes – 95% water (+0.1% TFA) / 5% acetonitrile (+0.05% TFA) down to 5% water / 95% acetonitrile. Flow rate 0.8mL/min [Method A]; or on a Hichrom 3.5 C18 reverse phase column (100mm x 3.2mm i.d.). Linear gradient elution over 11 minutes – 95% water (+0.1% TFA) / 5% acetonitrile (+0.05% TFA) down to 5% water / 95% acetonitrile. Flow rate 1mL/min [Method B]. Samples were routinely monitored at 254nM unless otherwise stated.

All reagents were purchased from commercial sources.

Experiment 1

Preparation and Analysis of 103487

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{(4-trifluoromethoxy)phenyl}amino] carboxamide

This compound is commercially available from Maybridge Chemical Company, Catalog No. KM04515.

Experiment 2

Preparation and Analysis of 116100

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][4-methoxyphenoxy]carboxamide

To 4-methoxyphenylchloroformate (19mg, 0.10mmol) in CH_2Cl_2 (0.5mL) was added dropwise a solution of 3-(3-aminophenyl)-4-bromo-1-methylpyrazole (25mg, 0.10mmol) and triethylamine (14μL, 0.10mmol) in CH_2Cl_2 (0.5mL). The mixture was stirred for 16 h and

concentrated. Chromatography on flash silica (40% EtOAc/hexane) gave the title compound as a colourless solid (21mg, 52%), m.p. 140.3-141.8°C (EtOAc/hexane).

IR: ν_{max} = 1748, 1592, 1504, 1412, 1190, 835, 764, 676 cm^{-1}. MS (ES+): *m/z* (%) = 404 (M+H ^{81}Br, 100), 402 (M+H ^{79}Br, 90).

^{1}H-NMR (CD_3OD): δ = 3.80 (3H, s, CH_3), 3.81 (3H, s, CH_3), 6.91-6.98 (2H, m, ArH), 7.07-7.18 (3H, m, ArH), 7.42-7.53 (4H, m, ArH). HPLC: retention time 3.28 mins [Method A]. Tlc : Rf 0.4 (EtOAc/hexane).

Experiment 3

Preparation and Analysis of 116101

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][4-trifluoromethoxyphenyl]carboxamide

To 4-(trifluoromethoxy)benzoyl chloride (19µL, 0.12mmol) in CH_2Cl_2 (1mL) was added dropwise a solution of 3-(3-aminophenyl)-4-bromo-1-methylpyrazole (30mg, 0.12mmol) and triethylamine (17µL, 0.12mmol) in CH_2Cl_2 (0.5mL). The reaction mixture was stirred for 16 h and concentrated. Chromatography on flash silica (50% EtOAc/hexane) gave the title compound as a colourless solid (40mg, 76%), m.p. 138.6-139.6°C (EtOAc/hexane).

MS (ES+): *m/z* (%) = 442 (M+H ^{81}Br, 93), 440 (M+H ^{79}Br, 100).

^{1}H-NMR (DMSO d_6): δ = 3.79 (3H, s, CH_3), 7.27 (1H, m, ArH), 7.45-7.60 (3H, m, ArH), 7.65 (1H, s, ArH), 7.87 (2H, m, ArH), 8.09 (2H, m, ArH), 10.51 (1H, s, NH).

HPLC: retention time 3.60 min [Method A]. TLC: Rf 0.40 (50% EtOAc/hexane).

Experiment 4

Preparation and Analysis of 116102

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][2-thienyl]carboxamide

To thiophene-2-carbonyl chloride (11µL, 0.09mmol) in CH_2Cl_2 (1mL) was added dropwise a solution of 3-(3-aminophenyl)-4-bromo-1-methylpyrazole (25mg, 0.09mmol) and triethylamine (14µL, 0.09mmol) in CH_2Cl_2 (0.5mL). The reaction mixture was stirred for 16 h and concentrated. Chromatography on flash silica (50% EtOAc/hexane) gave the title compound as a colourless solid (24mg, 68%), m.p. 127.8-128.6°C (EtOAc/hexane).

MS (ES+): *m/z* (%) = 364 (M+H ^{81}Br, 96), 362 (M+H ^{79}Br, 100).

^{1}H-NMR (CD_3OD): δ = 3.81 (3H, s, CH_3), 7.19 (2H, m, ArH), 7.48-7.58 (2H, m, ArH), 7.68-7.83 (3H, m, ArH), 7.93 (1H, dd, J=1.0, 3.8, ArH).

HPLC: retention time 3.12 min [Method A]. TLC: Rf 0.30 (30% EtOAc/hexane).

Experiment 5

Preparation and Analysis of 116115

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{(4-trifluoromethoxy)phenyl)methyl}amino]carboxamide

To a stirred solution of triphosgene (12mg, 0.04mmol) in CH_2Cl_2 (0.5mL) was added dropwise a solution of 3-(3-aminophenyl)-4-bromo-1-methylpyrazole (30mg, 0.12mmol) and triethylamine (33μL, 0.24mmol) in CH_2Cl_2 (0.5mL). After 1 h, 4-(trifluoromethoxy)benzylamine (23mg, 0.12mmol) was added. The reaction mixture was stirred for 16 h and concentrated. Chromatography on flash silica (75%EtOAc/hexane) gave the title compound as a colourless solid (38mg, 68%), m.p. 144.6-145.8°C (EtOAc/hexane).

IR: ν_{max} = 1626, 1558, 1278, 1160, 969, 871, 789, 703 cm^{-1}. MS (ES+): *m/z* (%) = 471 (M+H ^{81}Br, 91), 469 (M+H ^{79}Br, 100).

^{1}H-NMR (CD_3OD): δ = 3.81 (3H, s, CH_3), 4.42 (2H, s, CH_2), 7.06 (1H, d, J=7.1, ArH), 7.24 (2H, d, J=8.4, ArH), 7.37-7.52 (6H, m, ArH). HPLC: retention time 3.06 mins [Method A]. Tlc: Rf 0.5 (EtOAc).

Experiment 6

Preparation and Analysis of 116120

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][4-chlorophenyl]carboxamide

To 4-chlorobenzoyl chloride (15mg, 0.08mmol) in CH_2Cl_2 (1mL) was added dropwise a solution of 3-(3-aminophenyl)-4-bromo-1-methylpyrazole (21mg, 0.08mmol) and triethylamine (12μL, 0.08mmol) in CH_2Cl_2 (0.5mL). The mixture was stirred for 16 h and concentrated. Chromatography on flash silica (50% EtOAc/hexane) gave the title compound as a colourless solid (23mg, 72%), m.p. 184.4-184.8°C (EtOAc/hexane).

MS (ES+): *m/z* (%) = 394 (M+H ^{81}Br ^{37}Cl, 34), 392 (M+H ^{79}Br ^{37}Cl (^{81}Br ^{35}Cl), 100), 390 (M+H ^{79}Br ^{35}Cl, 67).

^{1}H-NMR (DMSO d_6): δ = 3.79 (3H, s, CH_3), 7.25 (1H, d, J=7.9, ArH), 7.51-7.65 (3H, m, ArH), 7.69 (1H, s, ArH), 7.90 (2H, m, ArH), 8.00 (2H, m, ArH), 10.51 (1H, s, NH).

HPLC: retention time 3.40 min [Method A]. TLC: Rf 0.35 (50% EtOAc/hexane).

Experiment 7

Preparation and Analysis of 116137

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]-2-[4-(trifluoromethoxy)phenyl]acetamide

A solution of 3-(3-aminophenyl)-4-bromo-1-methylpyrazole (35mg, 0.14mmol) and triethylamine (23μL, 0.17mmol) in DMF (0.5mL) was added in one portion to a stirred solution of 4-trifluoromethoxyphenylacetic acid (31mg, 0.14mmol), HBTU (53mg, 0.14mmol) and HOBT (19mg, 0.14mmol) in DMF (1mL). The mixture was heated at 70°C for 24 h and then quenched with aqueous sodium bicarbonate solution. Ethyl acetate was added and the organic phase separated, washed with water (x3), brine, dried ($MgSO_4$) and evaporated. Chromatography on flash silica (50%EtOAc/hexane) gave the title compound as a colourless solid (43mg, 68%), m.p. 141.2-142.5°C (EtOAc/hexane).

IR: ν_{max} = 1684, 1592, 1510, 1253, 1217, 1157, 987, 798, 700 cm^{-1}.

MS (ES+): *m/z* (%) = 456 (M+H ^{81}Br, 100), 454 (M+H ^{79}Br, 94).

^{1}H-NMR (DMSO d_6): δ = 3.72 (2H, s, CH_2), 3.75 (3H, s, CH_3), 7.17 (1H, d, J=7.7, ArH), 7.33 (2H, d, J=8.7, ArH), 7.38-7.51 (3H, m, ArH), 7.62-7.73 (3H, m, ArH), 10.44 (1H, s, NH).

HPLC: retention time 3.52 min [Method A].

Experiment 8

Preparation and Analysis of 116174

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]-2-(3-fluorophenyl)acetamide

A mixture of 3-(3-aminophenyl)-4-bromo-1-methylpyrazole (30 mg, 0.12 mmol), 3-fluorophenylacetic acid (18 mg, 0.12 mmol), 1-hydroxybenzotriazole hydrate (16 mg, 0.12 mmol) and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate (46 mg, 0.12 mmol) were dissolved in chloroform (1.5 ml). N, N-Diisopropylethylamine (0.02 ml, 0.13 mmol) was added and the mixture stirred at room temperature for 16h. The reaction mixture was then poured into brine and the organic layer washed with further brine, dried over magnesium sulphate and then concentrated in vacuo. The crude product was purified by column chromatography (ethyl acetate-toluene, 1:1), giving the title compound (12 mg, 26 %). Rf 0.41 (ethyl acetate-toluene, 1:1).

HPLC (Method B): retention time 7.07 min (100 %). δ_H ($CDCl_3$) 3.77 (2H, s), 3.83 (3H, s), 7.02 – 7.20 (4H, m), 7.54 (1H, s), 7.60 – 7.63 (1H, m). MS (AP+): *m/z* (%) = 390 (M + H ^{81}Br, 100), 388 (M + H ^{79}Br, 100).

Experiment 9

Preparation and Analysis of 116175

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]-2-(3-methoxyphenyl)acetamide

A solution of 3-methoxyphenylacetyl chloride (0.02 ml, 0.12 mmol) in dichloromethane (0.75 ml) was added dropwise at 0 °C to a solution of 3-(3-aminophenyl)-4-bromo-1-methylpyrazole (30 mg, 0.12 mmol) and triethylamine (0.02 ml, 0.13 mmol) in dichloromethane (0.75 ml). The resulting mixture was stirred at room temperature for 16h and then poured into brine. The organic layer was washed with more brine then dried over magnesium sulphate and concentrated *in vacuo*. The crude product was purified by column chromatography (ethyl acetate-toluene, 1:1), giving the title compound (9 mg, 19 %). Rf 0.30 (ethyl acetate-toluene, 1:1).

HPLC (Method B): retention time 8.62 min (97.09 %). δ_H ($CDCl_3$) 3.76 (2H, s), 3.82 (3H, s), 3.85 (3H, s), 6.84 – 6.90 (3H, m), 7.07 – 7.44 (5H, m), 7.53 (1H, s), 7.60 (1H, br s). MS (AP+): *m/z* (%) = 402 (M + H ^{81}Br, 100), 400 (M + H ^{79}Br, 95).

Experiment 10

Preparation and Analysis of 116176

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]-2-(2-fluorophenyl)acetamide

A mixture of 3-(3-aminophenyl)-4-bromo-1-methylpyrazole (30 mg, 0.12 mmol), 2-fluorophenylacetic acid (18 mg, 0.12 mmol), 1-hydroxybenzotriazole hydrate (16 mg, 0.12 mmol) and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate (46 mg, 0.12 mmol) were dissolved in chloroform (1.5 ml). N, N-Diisopropylethylamine (0.02 ml, 0.13 mmol) was added and the mixture stirred at room temperature for 16h. The reaction mixture was then poured into brine and the organic layer washed with further brine, dried over magnesium sulphate and then concentrated in vacuo. The crude product was purified by column chromatography (ethyl acetate-toluene, 1:1), giving the title compound (15 mg, 32 %). Rf 0.52 (ethyl acetate-toluene, 1:1).

HPLC (Method B): retention time 7.28 min (100 %). δ_H ($CDCl_3$) 3.79 (2H, s), 3.83 (3H, s), 7.11 – 7.23 (3H, m), 7.30 – 7.55 (6H, m), 7.61 – 7.64 (1H, m). MS (AP+): *m/z* (%) = 390 (M + H ^{81}Br, 100), 388 (M + H ^{79}Br, 100).

Experiment 11

Preparation and Analysis of 116177

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]-2-(4-nitrophenyl)acetamide

A mixture of 3-(3-aminophenyl)-4-bromo-1-methylpyrazole (30 mg, 0.12 mmol), 4-nitrophenylacetic acid (22 mg, 0.12 mmol), 1-hydroxybenzotriazole hydrate (16 mg, 0.12 mmol) and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (46 mg, 0.12 mmol) were dissolved in chloroform (1.5 ml). N, N-Diisopropylethylamine (0.02 ml, 0.13 mmol) was added and the mixture stirred at room temperature for 16h. The reaction mixture was then poured into brine and the organic layer washed with further brine, dried over magnesium sulphate and then concentrated in vacuo. The crude product was purified by column chromatography (ethyl acetate-toluene, 1:1), giving the title compound (9 mg, 18 %). Rf 0.19 (ethyl acetate-toluene, 1:1).

HPLC (Method B): retention time 7.22 min (94.30 %). δ_H ($CDCl_3$) 3.83 (3H, s), 3.87 (2H, s), 7.18 – 7.23 (1H, m), 7.42 – 7.65 (7H, m), 8.22 – 8.30 (2H, m). MS (AP+): *m/z* (%) = 417 (M + H ^{81}Br, 100), 415 (M + H ^{79}Br, 100).

Experiment 12

Preparation and Analysis of 116178

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]-2-(2-methoxyphenyl)acetamide

A mixture of 3-(3-aminophenyl)-4-bromo-1-methylpyrazole (30 mg, 0.12 mmol), 2-methoxyphenylacetic acid (20 mg, 0.12 mmol), 1-hydroxybenzotriazole hydrate (16 mg, 0.12 mmol) and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (46 mg, 0.12 mmol) were dissolved in chloroform (1.5 ml). N, N-Diisopropylethylamine (0.02 ml, 0.13 mmol) was added and the mixture stirred at room temperature for 16h. The reaction mixture was then poured into brine and the organic layer washed with further brine, dried over magnesium sulphate and then concentrated in vacuo. The crude

product was purified by column chromatography (chloroform-methanol, 99:1), giving the title compound (18 mg, 38 %) as a colourless solid. Rf 0.65 (chloroform-methanol, 98:2).

HPLC (Method B): retention time 7.16 min (100 %). δ_H ($CDCl_3$) 3.76 (2H, s), 3.83 (3H, s), 3.98 (3H, s), 6.97 – 7.06 (2H, m), 7.11 – 7.16 (1H, m), 7.31 – 7.50 (4H, m), 7.53 (1H, s), 7.57 – 7.60 (1H, m), 7.91 (1H, br s). MS (AP-): *m/z* (%) = 400 (M – H ^{81}Br, 90), 398 (M – H ^{79}Br, 100).

Experiment 13

Preparation and Analysis of 116192

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(1,1-dimethylethoxy)carboxamide

To di-*tert*-butyl dicarbonate (36mg, 0.17mmol) in methanol (1mL) was added dropwise a solution of 3-(3-aminophenyl)-4-bromo-1-methylpyrazole (42mg, 0.17mmol) in methanol (1mL). The mixture was stirred for 16 h and concentrated. Chromatography on flash silica (40%EtOAc/heaxne) gave the title compound as a colourless solid (29mg, 49%) (EtOAc/hexane).

MS (CI-): *m/z* (%) = 352 (M-H ^{81}Br, 100), 350 (M-H ^{79}Br, 96).

^{1}H-NMR (DMSO d_6): δ = 1.46 (9H, s, 3xCH_3), 3.73 (3H, s, CH_3), 7.07 (1H, m, ArH), 7.42 (1H, t, J=7.7, ArH), 7.53-7.60 (2H, m, ArH), 7.64 (1H, s, ArH), 9.57 (1H, s, NH).

HPLC: retention time 7.15 min [Method B].

One or the other (as indicated) of the two following synthetic protocols was used to generate each of the compounds below:

Protocol A:

To an isocyanate (1mmol) in CH_2Cl_2 (4mL) was added dropwise a solution of 3-(3-aminophenyl)-4-bromo-1-methylpyrazole (1mmol) in CH_2Cl_2 (4mL). The mixture was stirred for 16 hours and concentrated. Chromatography on flash silica (20%-80% EtOAc/hexane) followed by recrystallisation gave the pure urea.

Protocol B:

To a stirred solution of triphosgene (0.33mmol) in CH_2Cl_2 (4mL) was added dropwise a solution of 3-(3-aminophenyl)-4-bromo-1-methylpyrazole (1mmol) and triethylamine (2mmol) in CH_2Cl_2 (4mL). After 1 hour, an aniline was added (1mmol). The

reaction mixture was stirred for 16 hours and concentrated. Chromatography on flash silica (20%-80%EtOAc/hexane) followed by recrystallisation gave the pure urea.

Experiment 14

Preparation and Analysis of 116079

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][(4-methylthiophenyl)amino]carboxamide

[Protocol A] – 4-(methylthio)phenyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 419 (M+H ^{81}Br, 100), 417 (M+H ^{79}Br, 94).

^1H-NMR (MeOH d_4): δ = 2.42 (3H, s, SCH_3), 3.81 (3H, s, NCH_3), 7.06 (1H, m, ArH), 7.22 (2H, m, ArH), 7.37 (2H, m, ArH), 7.42-7.61 (4H, m, ArH).

HPLC: retention time 3.35 min [Method A].

Experiment 15

Preparation and Analysis of 116081

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][(4-chlorophenyl)amino]carboxamide

[Protocol A] – 4-chlorophenyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 409 (M+H ^{81}Br ^{37}Cl, 19), 407 (M+H ^{79}Br ^{37}Cl (^{81}Br ^{35}Cl), 100), 405 (M+H ^{79}Br ^{35}Cl, 81).

^1H-NMR (MeOH d_4): δ = 3.81 (3H, s, CH_3), 7.07 (1H, m, ArH), 7.23 (2H, m, ArH), 7.36-7.60 (6H, m, ArH).

HPLC: retention time 3.42 min [Method A].

Experiment 16

Preparation and Analysis of 1160°2

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(4-fluorophenyl)carboxamide

[Protocol A] – 4-fluorophenyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 391 (M+H ^{81}Br, 96), 389 (M+H ^{79}Br, 100).

^{1}H-NMR (MeOH d_4): δ = 3.81 (3H, s, CH_3), 6.93-7.11 (3H, m, ArH), 7.37-7.61 (6H, m, ArH).

HPLC: retention time 3.11 min.

Experiment 17

Preparation and Analysis of 116087

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[2-(trifluoromethoxy)phenyl]carboxamide

[Protocol A] - 2-(trifluoromethoxy)phenyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 457 (M+H ^{81}Br, 100), 455 (M+H ^{79}Br, 95).

^{1}H-NMR (DMSO d_6): δ = 3.79 (3H, s, CH_3), 7.06-7.18 (2H, m, ArH), 7.38-7.49 (2H, m, ArH), 7.51-7.62 (2H, m, ArH), 7.65 (1H, m, ArH), 7.71 (1H, s, ArH), 8.24 (1H, dd, J=1.1, 8.2, ArH), 8.56 (1H, s, NH), 9.49 (1H, s, NH).

HPLC: retention time 3.40 min.

Experiment 18

Preparation and Analysis of 116089

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-nitrophenyl)carboxamide

[Protocol A] - 2-nitrophenyl isocyanate

yellow solid (EtOAc/hexane)

MS (ES+): m/z (%) = 418 (M+H ^{81}Br, 98), 416 (M+H ^{79}Br, 100).

^{1}H-NMR (DMSO d_6): δ = ^{1}H-NMR (DMSO d_6): □ = 3.79 (3H, s, NCH_3), 7.14 (1H, m, ArH), 7.24 (1H, m, ArH), 7.50 (1H, t, J=7.7, ArH), 7.60 (2H, m, ArH), 7.67 (1H, s, ArH), 7.71 (1H, s, ArH), 8.10 (1H, m, ArH), 8.29 (1H, m, ArH), 9.65 (1H, s, NH), 10.09 (1H, s, NH).

HPLC: retention time 3.10 min [Method A].

Experiment 19

Preparation and Analysis of 116091

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(4-methoxyphenyl)carboxamide

[Protocol A] – 4-methoxyphenyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 403 (M+H ^{81}Br, 100), 401 (M+H ^{79}Br, 96).

^{1}H-NMR (DMSO d_6): δ = 3.71 (3H, s, OCH_3), 3.79 (3H, s, NCH_3), 6.87 (2H, d, J=8.9, ArH), 7.06 (1H, d, J=7.5, ArH), 7.39 (2H, d, J=8.9, ArH), 7.45-7.61 (3H, m, ArH), 7.65 (1H, s, ArH), 8.52 (1H, s, NH), 8.84 (1H, s, NH).

HPLC: retention time 3.08 min.

Experiment 20

Preparation and Analysis of 116092

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-methylphenyl)carboxamide

[Protocol A] – *o*-tolyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 387 (M+H ^{81}Br, 94), 385 (M+H ^{79}Br, 100).

^{1}H-NMR (MeOH d_4): δ = 2.29 (3H, s, CH_3), 3.81 (3H, s, NCH_3), 7.03 (1H, dt, J=1.1,7.5, ArH), 7.09 (1H, dt, J=1.1, 7.5, ArH), 7.13-7.22 (2H, m, ArH), 7.45 (1H, t, J=7.9, ArH), 7.49-7.57 (2H, m, ArH), 7.60-7.68 (2H, m, ArH).

HPLC: retention time 2.96 min.

Experiment 21

Preparation and Analysis of 116097

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[4-(trifluoromethyl)phenyl]carboxamide

[Protocol A] – 4-(trifluoromethyl)phenyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 441 (M+H ^{81}Br, 94), 439 (M+H ^{79}Br, 100).

^{1}H-NMR (MeOH d_4): δ = 3.82 (3H, s, CH_3), 7.04-7.16 (3H, m, ArH), 7.20-7.47 (6H, m, ArH).

HPLC: retention time 3.56 min.

Experiment 22

Preparation and Analysis of 116105

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(3-chlorophenyl)carboxamide

[Protocol A] – 3-chlorophenyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): *m/z* (%) = 409 (M+H ^{81}Br ^{37}Cl, 26), 407 (M+H ^{79}Br ^{37}Cl (^{81}Br ^{35}Cl), 100), 405 (M+H ^{79}Br ^{35}Cl, 70).

^{1}H-NMR (MeOH d_4): δ = 3.81 (3H, s, NCH_3), 7.04 (1H, m, ArH), 7.10 (1H, m, ArH), 7.28 (2H, m, ArH), 7.47 (1H, t, J=7.8, ArH), 7.55 (1H, m, ArH), 7.63 (1H, m, ArH), 7.68 (1H, s, ArH), 7.73 (1H, m, ArH), 9.04 (2H, s, NH).

HPLC: retention time 3.20 min [Method A].

Experiment 23

Preparation and Analysis of 116108

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-chlorophenyl)carboxamide

[Protocol A] – 2-chlorophenyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): *m/z* (%) = 409 (M+H ^{81}Br ^{37}Cl, 24), 407 (M+H ^{79}Br ^{37}Cl (^{81}Br ^{35}Cl), 100), 405 (M+H ^{79}Br ^{35}Cl, 72).

^{1}H-NMR (MeOH d_4): δ = 3.81 (3H, s, NCH_3), 7.03 (1H, m, ArH), 7.11 (1H, m, ArH), 7.28 (1H, m, ArH), 7.35-7.53 (3H, m, ArH), 7.55 (1H, s, ArH), 7.62 (1H, m, ArH), 8.11 (1H, m, ArH).

HPLC: retention time 3.13 min.

Experiment 24

Preparation and Analysis of 116110

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[4-(methylethyl)phenyl]carboxamide

[Protocol A] – 4-isopropylphenyl isocyanate

colourless solid (THF/hexane)

MS (ES+): *m/z* (%) = 415 (M+H ^{81}Br, 100), 413 (M+H ^{79}Br, 92).

^{1}H-NMR (MeOH d_4): δ = 1.23 (6H, d, J=6.8, 2xCH_3), 2.86 (1H, septet, J=6.8, CH), 3.82 (3H, s, NCH_3), 7.09 (1H, m, ArH), 7.16 (2H, d, J=7.6, ArH), 7.31 (2H, d, J=7.6, ArH), 7.42-7.51 (2H, m, ArH), 7.54 (1H, s, ArH), 7.59 (1H, m, ArH).

HPLC: retention time 3.66 min.

Experiment 25

Preparation and Analysis of 116111

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(3-methoxyphenyl)carboxamide

[Protocol A] – 3-methoxyphenyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): *m/z* (%) = 403 (M+H ^{81}Br, 100), 401 (M+H ^{79}Br, 96).

^{1}H-NMR (MeOH d_4): δ = 3.73 (3H, s, OCH_3), 3.81 (3H, s, NCH_3), 6.59 (1H, m, ArH), 6.91 (1H, m, ArH), 7.08 (1H, m, ArH), 7.14 (2H, m, ArH), 7.39-7.61 (4H, m, ArH).

HPLC: retention time 2.90 min.

Experiment 26

Preparation and Analysis of 116112

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(3-methylphenyl)carboxamide

[Protocol A] – *m*-tolyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): *m/z* (%) = 387 (M+H ^{81}Br, 100), 385 (M+H ^{79}Br, 96).

^{1}H-NMR (DMSO d_6): δ = 2.26 (3H, s, CH_3), 3.76 (3H, s, NCH_3), 6.79 (1H, m, ArH), 7.06-7.22 (3H, m, ArH), 7.29 (1H, m, ArH), 7.43-7.62 (3H, m, ArH), 7.68 (1H, s, ArH), 8.65 (1H, s, NH), 8.89 (1H, s, NH).

HPLC: retention time 3.05 min [Method A].

Experiment 27

Preparation and Analysis of 116113

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-methyl-N-[4-(trifluoromethoxy)phenyl]carboxamide

[Protocol B] – *N*-methyl-4-(trifluoromethoxy)aniline

pale yellow solid (EtOAc/hexane)

MS (ES+): *m/z* (%) = 471 (M+H ^{81}Br, 88), 469 (M+H ^{79}Br, 100).

^{1}H-NMR (MeOH d_4): δ = 3.35 (3H, s, NCH_3), 3.81 (3H, s, NCH_3), 7.09 (1H, m, ArH), 7.25-7.51 (8H, m, ArH).

HPLC: retention time 3.56 min [Method A].

Experiment 28

Preparation and Analysis of 116119

N-[4-(*tert*-butyl)phenyl]{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}carboxamide

[Protocol B] – 4-*tert*-butylaniline

colourless solid (EtOAc/hexane)

MS (ES+): *m/z* (%) = 429 (M+H ^{81}Br, 98), 427 (M+H ^{79}Br, 100).

^{1}H-NMR (DMSO d_6): δ = 1.27 (9H, s, 3xCH_3), 3.79 (3H, s, NCH_3), 7.07 (1H, d, J=7.5, ArH), 7.29 (2H, d, J=8.7, ArH), 7.37 (2H, d, J=8.7, ArH), 7.45 (1H, t, J=7.5, ArH), 7.51-7.60 (2H, m, ArH), 7.66 (1H, s, ArH), 8.65 (1H, s, NH), 8.83 (1H, s, NH).

HPLC: retention time 3.77 min.

Experiment 29

Preparation and Analysis of 116122

N-[4-(dimethylamino)phenyl]{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}carboxamide

[Protocol B] – *N,N*-dimethyl-*p*-phenylenediamine

colourless solid (EtOAc/hexane)

MS (ES+): *m/z* (%) = 416 (M+H ^{81}Br, 96), 414 (M+H ^{79}Br, 100).

^{1}H-NMR (DMSO d_6): δ = 2.86 (6H, s, NCH_3), 3.80 (3H, s, NCH_3), 6.80 (2H, m, ArH), 7.09 (1H, d, J=7.7, ArH), 7.28 (2H, m, ArH), 7.42 (1H, t, J=7.8, ArH), 7.52 (1H, m, ArH), 7.59 (1H, s, ArH), 7.67 (1H, s, ArH), 8.45 (1H, s, NH), 8.75 (1H, s, NH).

HPLC: retention time 2.07 min [Method A].

Experiment 30

Preparation and Analysis of 116138

N-(3,5-dichloro-4-methylphenyl){[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}carboxamide

[Protocol B] – 3,5-dichloro-4-methylphenylamine

colourless solid (EtOAc/hexane)

MS (ES+): *m/z* (%) = 457 (M+H, 35), 455 (M+H, 100), 453 (M+H, 65).

^{1}H-NMR (DMSO d_6): δ = 2.32 (3H, s, CH_3), 3.79 (3H, s, NCH_3), 7.11 (1H, d, J=7.4, ArH), 7.46 (1H, t, J=7.8, ArH), 7.50-7.64 (4H, m, ArH), 7.68 (1H, s, ArH), 9.02 (1H, s, NH), 9.09 (1H, s, NH).

HPLC: retention time 3.66 min.

Experiment 31

Preparation and Analysis of 116139

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[4-(trifluoromethylthio)phenyl]carboxamide

[Protocol B] – 4-(trifluoromethylthio)aniline

colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 473 (M+H ^{81}Br, 100), 471 (M+H ^{79}Br, 94).

^{1}H-NMR (DMSO d_6): δ = 3.81 (3H, s, NCH_3), 7.11 (1H, d, J=7.5, ArH), 7.47 (1H, t, J=7.9, ArH), 7.51-7.63 (6H, m, ArH), 7.66 (1H, s, ArH), 9.03 (1H, s, NH), 9.16 (1H, s, NH).

HPLC: retention time 3.76 min.

Experiment 32

Preparation and Analysis of 116141

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(cyclohexyl)carboxamide

[Protocol B] – cyclohexylamine

colourless solid, m.p. 155.5-156.3°C (EtOAc/hexane).

MS (ES+): m/z (%) = 379 (M+H ^{81}Br, 93), 377 (M+H ^{79}Br, 100).

^{1}H-NMR (DMSO d_6): δ = 1.07-1.34 (5H, m, 5xCH), 1.52 (1H, m, CH), 1.63 (2H, m, 2xCH), 1.76 (2H, m, 2xCH), 3.48 (1H, m, NCH), 3.74 (3H, s, CH_3), 6.15 (1H, d, J=7.8, ArH), 6.98 (1H, d, J=7.5, ArH), 7.32-7.43 (2H, m, ArH), 7.51 (1H, m, NH), 7.62 (1H, s, ArH), 8.50 (1H, s, NH).

HPLC: retention time 3.16 min [Method A].

TLC: retention factor 0.35 (50% EtOAc/hexane).

Experiment 33

Preparation and Analysis of 116143

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(phenylmethyl)carboxamide

[Protocol B] – benzylamine

colourless solid, m.p. 144.5-146.2°C (EtOAc/hexane).

IR: $\square_{max}$ = 1622, 1565, 1467, 1374, 1239, 973, 802, 752, 695 cm^{-1}.

MS (ES+): m/z (%) = 387 (M+H ^{81}Br, 89), 385 (M+H ^{79}Br, 100).

^{1}H-NMR (CD_3OD): δ = 3.81 (3H, s, CH_3), 4.40 (2H, s, CH_2), 7.05 (1H, m, ArH), 7.19-7.51 (9H, m, ArH).

HPLC: retention time 3.06 min [Method A].a

Experiment 34

Preparation and Analysis of 116144

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-fluorophenyl)carboxamide

[Protocol A] – 2-fluorophenyl isocyanate

colourless solid (DCM/hexane)

MS (ES+): *m/z* (%) = 391 (M+H ^{81}Br, 100), 389 (M+H ^{79}Br, 90).

^{1}H-NMR (MeOH d_4): δ = 3.79 (3H, s, NCH_3), 7.00-7.11 (4H, m, ArH), 7.40-7.56 (3H, m, ArH), 7.61 (1H, m, ArH), 8.09 (1H, m, ArH).

HPLC: retention time 3.01 min.

Experiment 35

Preparation and Analysis of 116145

2-({[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}carbonylamino)benzamide

[Protocol B] – 2-aminobenzamide

colourless solid (EtOAc/hexane)

MS (ES+): *m/z* (%) = 399 (M+H -17 ^{81}Br, 100), 397 (M+H - 17 ^{79}Br, 94).

^{1}H-NMR (DMSO d_6): δ = 3.79 (3H, s, NCH_3), 6.93-7.10 (2H, m, ArH), 7.45 (2H, t, J=7.8, ArH), 7.59-7.72 (5H, m, ArH), 8.22 (2H, m), 9.92 (1H, s, NH), 10.69 (1H, s, NH).

HPLC: retention time 2.88 min.

Experiment 36

Preparation and Analysis of 116147

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(4-cyanophenyl)carboxamide

[Protocol B] – 4-aminobenzonitrile

colourless solid (EtOAc/hexane)

MS (ES+): *m/z* (%) = 398 (M+H ^{81}Br, 100), 396 (M+H ^{79}Br, 96).

^{1}H-NMR (MeOH d_4): δ = 3.81 (3H, s, NCH_3), 7.12 (1H, m, ArH), 7.46-7.57 (3H, m, ArH), 7.62-7.69 (5H, m, ArH).

HPLC: retention time 3.12 min.

Experiment 37

Preparation and Analysis of AR116148

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-cyanophenyl)carboxamide

[Protocol B] – 2-aminobenzonitrile

colourless solid (EtOAc/hexane)

MS (ES+): *m/z* (%) = 398 (M+H ^{81}Br, 95), 396 (M+H ^{79}Br, 100).

^{1}H-NMR ($CDCl_3$): δ = 3.79 (3H, s, CH_3), 7.13-7.28 (2H, m, ArH), 7.49 (1H, t, J=7.8, ArH), 7.57 (1H, m, ArH), 7.62 (1H, m, ArH), 7.65-7.71 (2H, m, ArH), 7.78 (1H, m, ArH), 8.07 (1H, d, J=8.6, ArH), 8.83 (1H, s, NH), 9.62 (1H, s, NH).

HPLC: retention time 3.05 min [Method A].

Experiment 38

Preparation and Analysis of 116182

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(4-fluorophenylmethyl)carboxamide

[Protocol B] – 4-fluorobenzylamine

colourless solid, m.p. 185.5-186.6°C (EtOAc/hexane).

MS (ES+): *m/z* (%) = 405 (M+H ^{81}Br, 97), 403 (M+H ^{79}Br, 100).

^{1}H-NMR (DMSO d_6): δ = 3.75 (3H, s, CH_3), 4.28 (2H, d, J=6.0, CH_2), 6.73 (1H, t, J=5.9, NH), 7.01 (1H, d, J=7.5, ArH), 7.10-7.18 (2H, m, ArH), 7.27-7.41 (4H, m, ArH), 7.56 (1H, s, ArH), 7.62 (1H, s, ArH), 8.82 (1H, s, NH).

HPLC: retention time 3.10 min [Method A].

TLC: retention factor 0.25 (50% EtOAc/hexane).

Experiment 39

Preparation and Analysis of 116183

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(3,4-dimethoxyphenylmethyl)carboxamide

[Protocol B] – 3,4-dimethoxybenzylamine

colourless solid, m.p. 174.9-175.5°C (EtOAc/hexane).

MS (CI+): *m/z* (%) = 447 (M+H ^{81}Br, 100), 445 (M+H ^{79}Br, 92).

^{1}H-NMR (DMSO d_6): δ = 3.71 (3H, s, CH_3), 3.73 (3H, s, CH_3), 3.76 (3H, s, CH_3), 4.22 (2H, d, J=5.8, CH_2), 6.62 (1H, t, J=5.7, NH), 6.80 (1H, m, ArH), 6.89 (2H, m, ArH), 6.98 (1H, m, ArH), 7.36-7.51 (3H, m, ArH), 7.63 (1H, s, ArH), 8.76 (1H, s, NH).

HPLC: retention time 2.86 min [Method A].

TLC: retention factor 0.20 (50% EtOAc/hexane).

Experiment 40

Preparation and Analysis of 116184

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(3,4,5-trimethoxyphenylmethyl)carboxamide

[Protocol B] – 3,4,5-trimethoxybenzylamine

colourless solid (EtOAc/hexane).

MS (CI+): *m/z* (%) = 477 (M+H ^{81}Br, 100), 475 (M+H ^{79}Br, 95).

^{1}H-NMR (DMSO d_6): δ = 3.63 (3H, s, OCH_3), 3.75 (9H, s, 3xCH_3), 4.21 (1H, d, J=5.9, CH_2), 6.61 (2H, s, ArH), 6.65 (1H, t, J=5.9, NH), 6.99 (1H, m, ArH), 7.40 (1H, t, J=7.7, ArH), 7.45 (1H, m, ArH), 7.56 (1H, m, ArH), 7.64 (1H, s, ArH), 8.77 (1H, s, NH).

HPLC: retention time 5.91 min [Method B].

TLC: retention factor 0.50 (50% EtOAc/hexane).

Experiment 41

Preparation and Analysis of 116185

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-methylphenylmethyl)carboxamide

[Protocol B] – 2-methylbenzylamine

colourless solid (EtOAc/hexane).

MS (CI+): *m/z* (%) = 401 (M+H ^{81}Br, 96), 399 (M+H ^{79}Br, 100).

^{1}H-NMR (DMSO d_6): δ = 2.28 (3H, s, CH_3), 3.76 (3H, s, NCH_3), 4.28 (1H, d, J=5.8, CH_2), 6.60 (1H, t, J=5.8, NH), 7.01 (1H, m, ArH), 7.15 (3H, m, ArH), 7.24 (1H, m, ArH), 7.38-7.50 (2H, m, ArH), 7.57 (1H, m, ArH), 7.65 (1H, s, ArH), 8.77 (1H, s, NH).

HPLC: retention time 2.74 min [Method A].

TLC: retention factor 0.20 (50% EtOAc/hexane).

Experiment 42

Preparation and Analysis of 116189

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(4-methoxyphenylmethyl)carboxamide

[Protocol B] – 4-methoxybenzylamine

colourless solid (EtOAc/hexane).

MS (CI+): *m/z* (%) = 417 (M+H ^{81}Br, 94), 415 (M+H ^{79}Br, 100).

^{1}H-NMR (DMSO d_6): δ = 3.72 (3H, s, CH_3), 3.77 (3H, s, NCH_3), 4.22 (1H, d, J=5.9, CH_2), 6.62 (1H, t, J=5.9, NH), 6.90 (2H, d, J=8.8, ArH), 7.00 (1H, m, ArH), 7.23 (2H, d,

J=8.8, ArH), 7.39 (1H, t, J=7.8, ArH), 7.43 (1H, m, ArH), 7.56 (1H, m, ArH), 7.64 (1H, s, ArH), 8.73 (1H, s, NH).

HPLC: retention time 6.41 min [Method B].

TLC: retention factor 0.25 (50% EtOAc/hexane).

Experiment 43

Preparation and Analysis of 116194

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[2-(4-methoxy)phenylethyl]carboxamide

[Protocol B] – 2-(4-methoxyphenyl)ethylamine

colourless solid (EtOAc/hexane).

MS (ES+): m/z (%) = 431 (M+H ^{81}Br, 95), 429 (M+H ^{79}Br, 100).

^{1}H-NMR (DMSO d_6): δ = 2.68 (2H, t, J=7.1, CH_2), 3.31 (2H, m, CH_2), 3.71 (3H, s, CH_3), 3.77 (3H, s, CH_3), 6.16 (1H, t, J=5.8, NH), 6.87 (2H, d, J=8.6, ArH), 6.99 (1H, dt, J=1.4, 7.3, ArH), 7.16 (2H, d, J=8.6, ArH), 7.33-7.48 (2H, m, ArH), 7.52 (1H, m, ArH), 7.63 (1H, s, ArH), 8.71 (1H, s, NH).

HPLC: retention time 6.62 min [Method B].

An important point that can be derived from the foregoing data is that by using a constitutively activated form of the receptor in the direct identification of candidate compounds, the selectivity of the compounds is exceptional: as those in the art appreciate, the homology between the human 5HT2A and 5HT2C receptors is about 95%, and even with such homology, certain of the directly identified compounds evidence a 4-order-of-magnitude (10,000-fold) selectivity separation (116100). This is important for pharmaceutical compositions in that such selectivity can help to reduce side-effects associated with interaction of a drug with a non-target receptor.

Different embodiments of the invention will consist of different constitutively activated receptors, different expression systems, different assays, and different compounds. Those skilled in the art will understand which receptors to use with which expression systems and assay methods. All are considered within the scope of the teaching of this invention. In addition, those skilled in the art will recognize that various modifications, additions, substitutions, and variations to the illustrative examples set forth herein can be made without departing from the spirit of the invention and are, therefore, considered within the scope of the invention.

CLAIMS

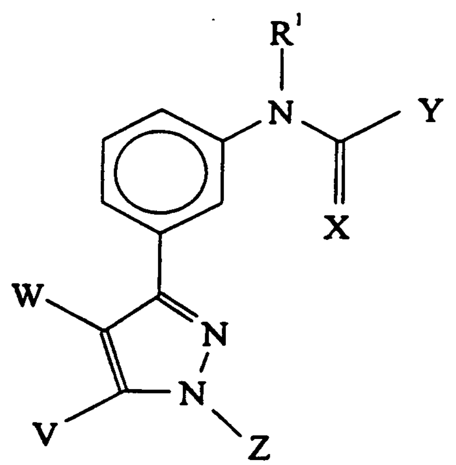
We claim:

1. A cDNA encoding a constitutively active, non-endogenous version, of a human $5HT_{2C}$ serotonin receptor comprising SEQ. ID NO. 28.
2. A constitutively active non-endogenous human $5HT_{2C}$ serotonin receptor encoded by the cDNA of SEQ ID NO. 28 comprising SEQ ID NO. 29.
3. A cDNA encoding a constitutively active, non-endogenous version, of a human $5HT_{2A}$ serotonin receptor comprising SEQ. ID NO. 30.
4. A constitutively active non-endogenous human $5HT_{2A}$ serotonin receptor encoded by the cDNA of SEQ ID NO. 30 comprising SEQ ID NO. 31.
5. A cDNA encoding a constitutively active, non-endogenous version, of a human $5HT_{2A}$ serotonin receptor comprising SEQ. ID NO. 32.
6. A constitutively active non-endogenous human $5HT_{2A}$ serotonin receptor encoded by the cDNA of SEQ ID NO. 32 comprising SEQ ID NO. 33.
7. A method for identifying whether a candidate compound is an inverse agonist to a non-endogenous human $5HT_2$ serotonin receptor comprising the steps of:
   a. contacting the candidate compound with a non-endogenous human $5HT_2$ serotonin receptor ; and
   b. determining, by measurement of a second messenger response whether said compound is an inverse agonist.
8. The method of claim 7 in which the non-endogenous human 5HT2 serotonin receptor comprises SEQ ID NO. 29.
9. The method of claim 7 in which the non-endogenous human 5HT2 serotonin receptor comprises SEQ ID NO. 31.
10. The method of claim 7 in which the non-endogenous human 5HT2 serotonin receptor comprises SEQ ID NO. 33.
11. An inverse agonist identified by the method of claim 7.
12. A reagent for screening compounds to determine whether the compounds are inverse agonists at human $5HT_2$ serotonin receptors comprising a membrane fraction from mammalian cells transfected with and expressing a cDNA encoding for a constitutively active, non-endogenous version, of a human $5HT_2$ serotonin receptor

in which the constitutively active non-endogenous human $5HT_2$ receptor is expressed on the cell surface.

13. A reagent for screening compounds to determine whether the compounds are inverse agonists at human $5HT_2$ serotonin receptors comprising mammalian cells which produce a second messenger response, transfected with and expressing a cDNA encoding for a constitutively active, non-endogenous version, of a human $5HT_2$ serotonin receptor in which the constitutively active non-endogenous human $5HT_2$ receptor is expressed on the cell surface.

14. A method for modulating by inverse agonism the activity of a human $5HT_{2A}$ serotonin receptor by contacting the receptor with a compound of formula:



(A)

Wherein:

W is lower alkyl (C_{1-6}), or halogen;
V is lower alkyl (C_{1-6}), or halogen;
X is either Oxygen or Sulfur;
Y is NR^2R^3, or $(CH_2)_mR^4$, or $O(CH_2)_nR^4$;
Z is lower alkyl (C_{1-6});
m = 0 - 4
n = 0 - 4
R^1 is H or lower alkyl (C_{1-4});
R^2 is H or lower alkyl(C_{1-4});

R^3 and R^4 are independently a C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, OCF_3, SMe, $COOR^7$, $SO_2NR^5R^6$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, and aryloxy wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, or aryloxy groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, $NHCOCH_3$, OCF3, SMe, $COOR^7$, SO_3R^7, $SO_2NR^5R^6$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl;

R^5 and R^6 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2 aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^7R^8$, NR^7R^8, $NHCOCH_3$, OCF_3, SMe, $COOR^9$, SO_3R^7, $SO_2NR^7R^8$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, or_aryl groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^8R^9$, NR^8R^9, $NHCOCH_3$, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H. halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl,

or R^5 and R^6 may form part of a 5, 6 or 7 membered cyclic structure which may be either saturated or unsaturated and that may contain up to four heteroatoms selected from O, N or S and said cyclic structure may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$. SO_3R^7, $NHCOCH_3$, COEt. COMe, or halogen;

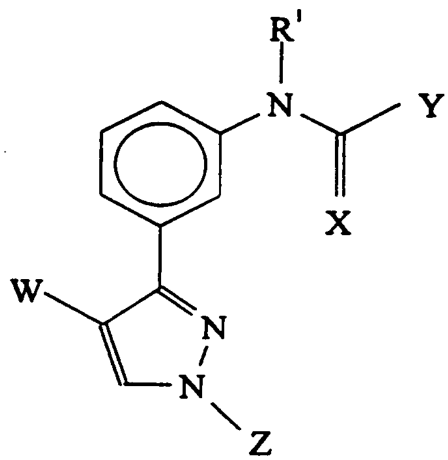
R^7 may be independently selected from H or C_{1-6} alkyl;

R^8 and R^9 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from

halogen, CF_3, OCF3, OEt, CCl_3, Me, NO_2, OH, OMe, SMe, COMe, CN, $COOR^7$, SO_3R^7, COEt, $NHCOCH_3$, or aryl;

an aryl moiety can be a 5 or 6 membered aromatic heterocyclic ring (containing up to 4 hetero atoms independently selected from N, O, or S) or a 6 membered aromatic non-heterocyclic ring or a polycycle;

15. A method for modulating by inverse agonism the activity of a human $5HT_{2A}$ serotonin receptor by contacting the receptor with a compound of formula:



(B)

Wherein:

W is Me, or Et, or halogen;

X is either Oxygen or Sulfur;

Y is NR^2R^3, or $(CH_2)_mR^4$, or $O(CH_2)_nR^4$;

Z is lower alkyl (C_{1-6});

m = 0 - 4

n = 0 - 4

R^1 is H or lower alkyl (C_{1-4});

R^2 is H or lower alkyl(C_{1-4});

R^3 and R^4 are independently a C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, OCF_3, SMe, $COOR^7$, $SO_2NR^5R^6$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6}

cycloalkyl, C_{1-6} alkyl, aryl, and aryloxy wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, or aryloxy groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, $NHCOCH_3$, OCF3, SMe, $COOR^7$, SO_3R^7, $SO_2NR^5R^6$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl;

R^5 and R^6 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2 aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^7R^8$, NR^7R^8, $NHCOCH_3$, OCF_3, SMe, $COOR^9$, SO_3R^7, $SO_2NR^7R^8$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, or_aryl groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^8R^9$, NR^8R^9, $NHCOCH_3$, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl,

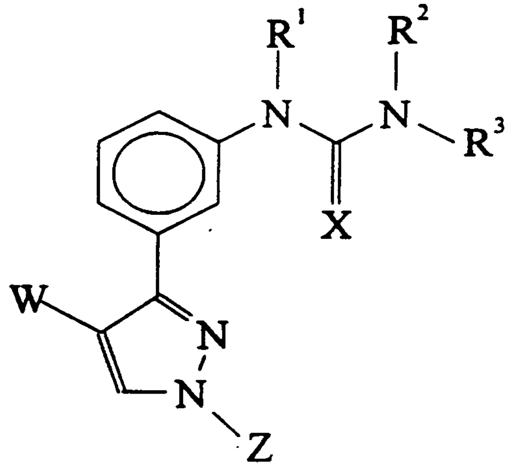
or R^5 and R^6 may form part of a 5, 6 or 7 membered cyclic structure which may be either saturated or unsaturated and that may contain up to four heteroatoms selected from O, N or S and said cyclic structure may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$, SO_3R^7, $NHCOCH_3$, COEt, COMe, or halogen;

R^7 may be independently selected from H or C_{1-6} alkyl;

R^8 and R^9 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from halogen, CF_3, OCF3, OEt, CCl_3, Me, NO_2, OH, OMe, SMe, COMe, CN, $COOR^7$, SO_3R^7, COEt, $NHCOCH_3$, or aryl;

an aryl moiety can be a 5 or 6 membered aromatic heterocyclic ring (containing up to 4 hetero atoms independently selected from N, O, or S) or a 6 membered aromatic non-heterocyclic ring or a polycycle.

16. A method for modulating by inverse agonism the activity of a human $5HT_{2A}$ serotonin receptor by contacting the receptor with a compound of formula:



(I)

Wherein:

Preferably R^1 and R^2 are H.

Preferably W is Br.

Preferably X is O.

Preferably Z is Me.

R^3 is C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, OCF_3, SMe, $COOR^7$, $SO_2NR^5R^6$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, and aryloxy wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, or aryloxy groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, $NHCOCH_3$, OCF3, SMe, $COOR^7$, SO_3R^7, $SO_2NR^5R^6$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl;

R^5 and R^6 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2 aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^7R^8$, NR^7R^8, $NHCOCH_3$, OCF_3, SMe.

$COOR^9$, SO_3R^7, $SO_2NR^7R^8$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, or_aryl groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^8R^9$, NR^8R^9, $NHCOCH_3$, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl,

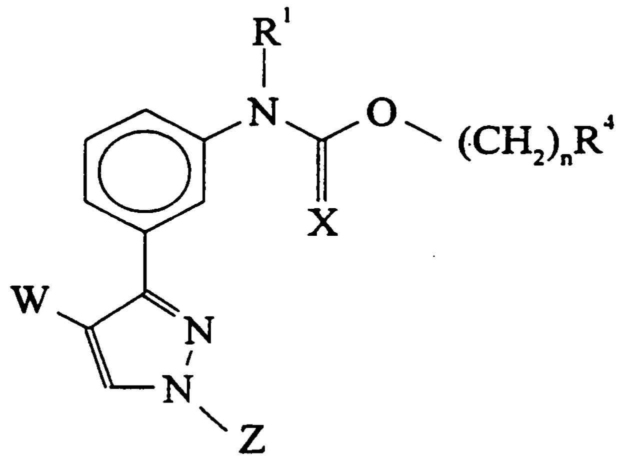
or R^5 and R^6 may form part of a 5, 6 or 7 membered cyclic structure which may be either saturated or unsaturated and that may contain up to four heteroatoms selected from O, N or S and said cyclic structure may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$, SO_3R^7, $NHCOCH_3$, COEt, COMe, or halogen;

R^7 may be independently selected from H or C_{1-6} alkyl;

R^8 and R^9 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from halogen, CF_3, OCF3, OEt, CCl_3, Me, NO_2, OH, OMe, SMe, COMe, CN, $COOR^7$, SO_3R^7, COEt, $NHCOCH_3$, or aryl;

an aryl moiety can be a 5 or 6 membered aromatic heterocyclic ring (containing up to 4 hetero atoms independently selected from N, O, or S) or a 6 membered aromatic non-heterocyclic ring or a polycycle.

17. A method for modulating by inverse agonism the activity of a human 5HT2A serotonin receptor by contacting the receptor with a compound of formula:



(II)

Wherein:

Preferably R^1 is H.

Preferably W is Br.

Preferably X is O.

Preferably Z is Me.

n = 0 - 4

R^4 is C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, OCF_3, SMe, $COOR^7$, $SO_2NR^5R^6$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, and aryloxy wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, or aryloxy groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$. NR^5R^6, $NHCOCH_3$, OCF3, SMe, $COOR^7$, SO_3R^7, $SO_2NR^5R^6$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl. C_{1-6} alkyl, and aryl;

R^5 and R^6 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl. or cycloalkyl, or aryl, or CH_2 aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^7R^8$, NR^7R^8, $NHCOCH_3$, OCF_3. SMe. $COOR^9$. SO_3R^7, $SO_2NR^7R^8$, COMe. COEt. CO-lower alkyl. SCF_3. CN. C_{2-6} alkenyl.

H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, or_aryl groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^8R^9$, NR^8R^9, $NHCOCH_3$, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl,

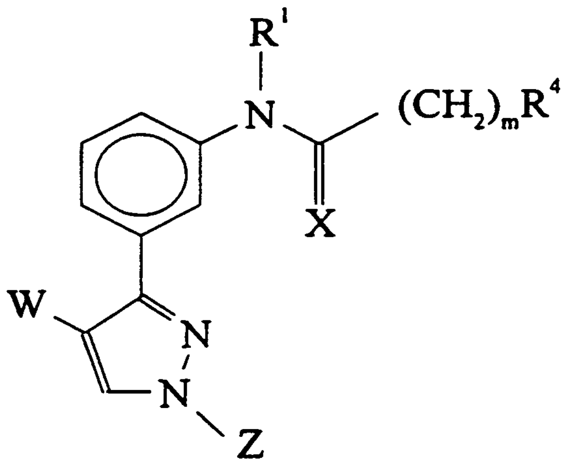
or R^5 and R^6 may form part of a 5, 6 or 7 membered cyclic structure which may be either saturated or unsaturated and that may contain up to four heteroatoms selected from O, N or S and said cyclic structure may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$, SO_3R^7, $NHCOCH_3$, COEt, COMe, or halogen;

R^7 may be independently selected from H or C_{1-6} alkyl;

R^8 and R^9 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from halogen, CF_3, OCF3, OEt, CCl_3, Me, NO_2, OH, OMe, SMe, COMe, CN, $COOR^7$, SO_3R^7, COEt, $NHCOCH_3$, or aryl;

an aryl moiety can be a 5 or 6 membered aromatic heterocyclic ring (containing up to 4 hetero atoms independently selected from N, O, or S) or a 6 membered aromatic non-heterocyclic ring or a polycycle.

18. A method for modulating by inverse agonism the activity of a human 5HT2A serotonin receptor by contacting the receptor with a compound of formula:



(III)

Wherein:

Preferably W is Br.

Preferably X is O.

Preferably Z is Me.

Preferably R^1 is H.

m = 0 - 4

R^4 is C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, OCF_3, SMe, $COOR^7$, $SO_2NR^5R^6$, SO_3R^7, COMe, COEt, CO-lower alkyl. SCF_3CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, and aryloxy wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, or aryloxy groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, $NHCOCH_3$, OCF3, SMe, $COOR^7$, SO_3R^7, $SO_2NR^5R^6$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl;

R^5 and R^6 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2 aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me. NO_2, OH, OMe, OEt, $CONR^7R^8$, NR^7R^8, $NHCOCH_3$. OCF_3, SMe. $COOR^9$. SO_3R^7. $SO_2NR^7R^8$, COMe, COEt, CO-lower alkyl, SCF_3, CN. C_{2-6} alkenyl.

H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, or_aryl groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^8R^9$, NR^8R^9, $NHCOCH_3$, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl,

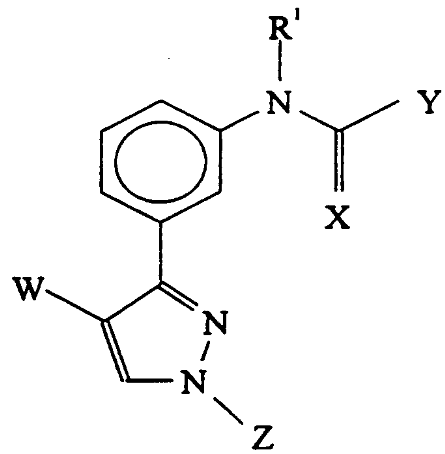
or R^5 and R^6 may form part of a 5, 6 or 7 membered cyclic structure which may be either saturated or unsaturated and that may contain up to four heteroatoms selected from O, N or S and said cyclic structure may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$, SO_3R^7, $NHCOCH_3$, COEt, COMe, or halogen;

R^7 may be independently selected from H or C_{1-6} alkyl;

R^8 and R^9 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from halogen, CF_3, OCF3, OEt, CCl_3, Me, NO_2, OH, OMe, SMe, COMe, CN, $COOR^7$, SO_3R^7, COEt, $NHCOCH_3$, or aryl;

an aryl moiety can be a 5 or 6 membered aromatic heterocyclic ring (containing up to 4 hetero atoms independently selected from N, O, or S) or a 6 membered aromatic non-heterocyclic ring or a polycycle.

19. A compound of formula (C)



(C)

Wherein:

W is Me, or Et, or halogen;

X is either Oxygen or Sulfur;

Y is NR^2R^3, or $(CH_2)_mR^4$, or $O(CH_2)_nR^4$;

Z is lower alkyl (C_{1-6});

m = 0 - 4;

n = 0 - 4;

R^1 is H or lower alkyl *(C_{1-4})*;

R^2 is H or lower alkyl(C_{1-4});

R^3 is a C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or $(CH_2)_k$aryl group (k = 1 – 4), preferably k = 1, and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH. OMe, OEt, $CONR^5R^6$, NR^5R^6, OCF_3, SMe, $COOR^7$, $SO_2NR^5R^6$, SO_3R^7, COMe. COEt, CO-lower alkyl, SCF_3CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, and aryloxy wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, or aryloxy groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me. NO_2. OH. OMe, OEt, $CONR^5R^6$, NR^5R^6, $NHCOCH_3$, OCF3, SMe, $COOR^7$. SO_3R^7.

$SO_2NR^5R^6$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl;

R^4 is a C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, OCF_3, SMe, $COOR^7$, $SO_2NR^5R^6$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, and aryloxy wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, aryl, or aryloxy groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^5R^6$, NR^5R^6, $NHCOCH_3$, OCF3, SMe, $COOR^7$, SO_3R^7, $SO_2NR^5R^6$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl;

R^5 and R^6 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2 aryl group and each said group may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^7R^8$, NR^7R^8, $NHCOCH_3$, OCF_3, SMe, $COOR^9$, SO_3R^7, $SO_2NR^7R^8$, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl wherein each of the C_{3-6} cycloalkyl, C_{1-6} alkyl, or_aryl groups may be further optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3, Me, NO_2, OH, OMe, OEt, $CONR^8R^9$, NR^8R^9, $NHCOCH_3$, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$, SO_3R^7, COMe, COEt, CO-lower alkyl, SCF_3, CN, C_{2-6} alkenyl, H, halogens, C_{1-4} alkoxy, C_{3-6} cycloalkyl, C_{1-6} alkyl, and aryl,

or R^5 and R^6 may form part of a 5, 6 or 7 membered cyclic structure which may be either saturated or unsaturated and that may contain up to four heteroatoms selected from O, N or S and said cyclic structure may be optionally substituted by up to four substituents in any position independently selected from CF_3, CCl_3. Me. NO_2, OH, OMe, OEt, OCF_3, SMe, $COOR^7$, $SO_2NR^8R^9$. SO_3R^7, $NHCOCH_3$, COEt. COMe, or halogen;

R^7 may be independently selected from H or C_{1-6} alkyl;

R^8 and R^9 are independently a H, or C_{1-6} alkyl, or C_{2-6} alkenyl, or cycloalkyl, or aryl, or CH_2aryl group and each said group may be optionally

substituted by up to four substituents in any position independently selected from halogen, CF_3, OCF3, OEt, CCl_3, Me, NO_2, OH, OMe, SMe, COMe, CN, $COOR^7$, SO_3R^7, COEt, $NHCOCH_3$, or aryl;

an aryl moiety can be a 5 or 6 membered aromatic heterocyclic ring (containing up to 4 hetero atoms independently selected from N, O, or S) or a 6 membered aromatic non-heterocyclic ring or a polycycle;

with the proviso that said compound is not:

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][methylamino]carboxamide, or

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{(4-trifluoromethoxy)phenyl}amino] carboxamide, or

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][2-chlorophenyl]carboxamide, or

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][2-chloro-3-pyridyl]carboxamide, or

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][trichloromethyl]carboxamide.

20. The use of a compound of claim 19 for the manufacture of a medicament.

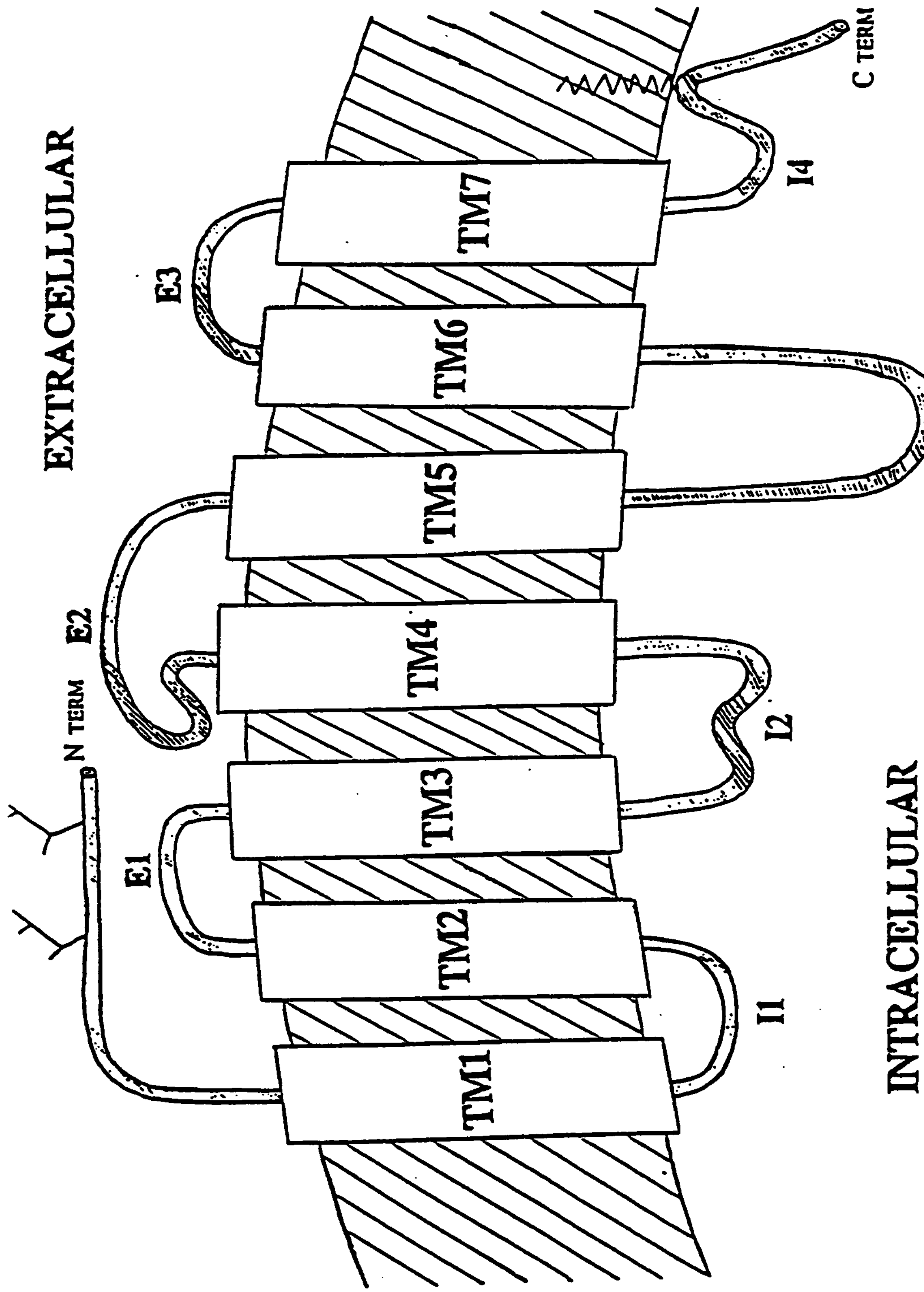

EXTRACELLULAR
INTRACELLULAR
N TERM
C TERM
TM1
TM2
TM3
TM4
TM5
TM6
TM7
E1
E2
E3
I1
I2
I3
I4

FIGURE 1

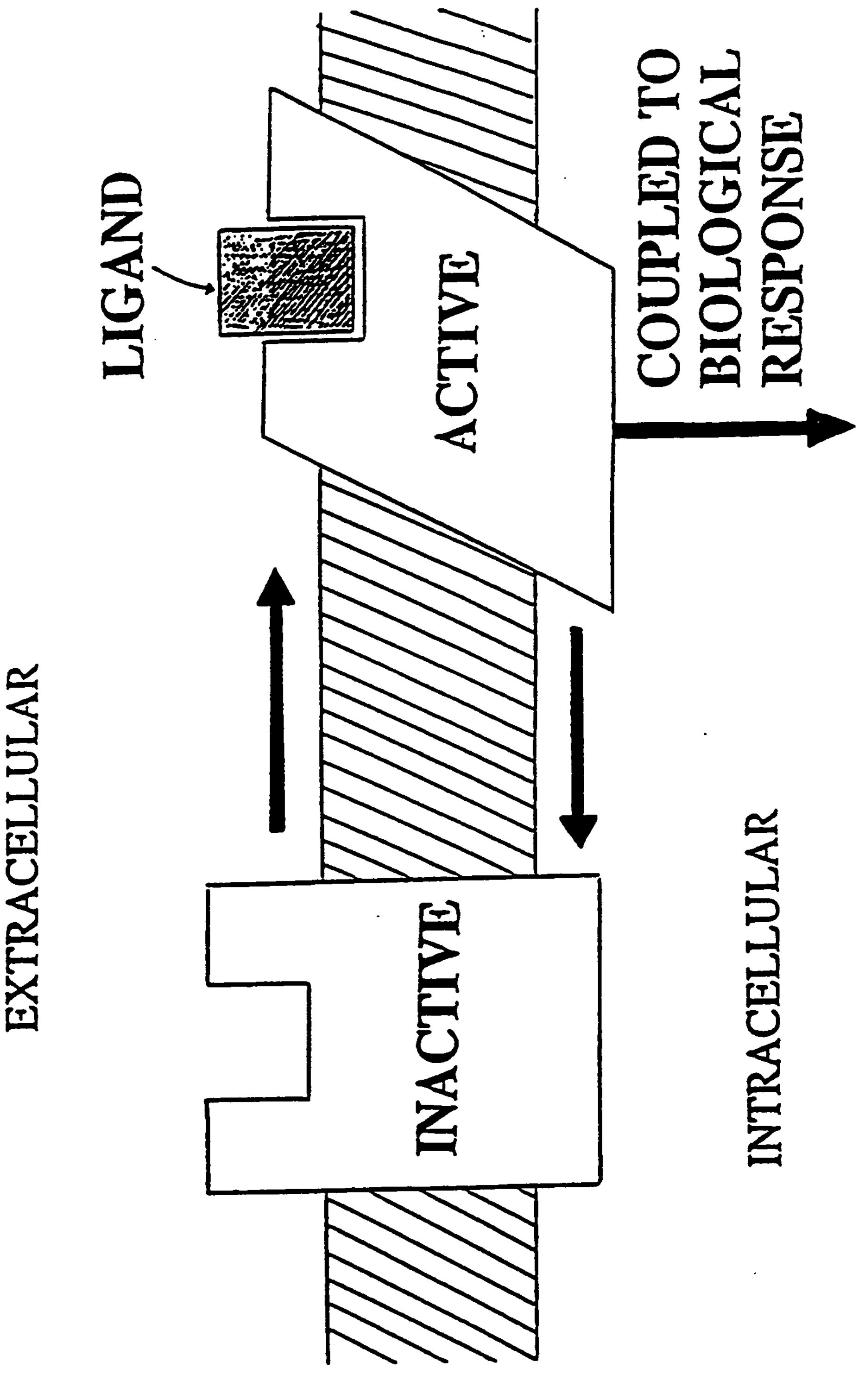

LIGAND
ACTIVE
COUPLED TO
BIOLOGICAL
RESPONSE
EXTRACELLULAR
INACTIVE
INTRACELLULAR

FIGURE 2

FIGURE 3A

ATGGATATTCTTTGTGAAGAAAATACTTCTTTGAGCTCAACTACGAACTCCCTAATGCAATTA
AATGATGACAACAGGCTCTACAGTAATGACTTTAACTCCGGAGAAGCTAACACTTCTGATGCA
TTTAACTGGACAGTCGACTCTGAAAATCGAACCAACCTTTCCTGTGAAGGGTGCCTCTCACCG
TCGTGTCTCTCCTTACTTCATCTCCAGGAAAAAAACTGGTCTGCTTTACTGACAGCCGTAGTGA
TTATTCTAACTATTGCTGGAAACATACTCGTCATCATGGCAGTGTCCCTAGAGAAAAAGCTGC
AGAATGCCACCAACTATTTCCTGATGTCACTTGCCATAGCTGATATGCTGCTGGGTTTCCTTGT
CATGCCCGTGTCCATGTTAACCATCCTGTATGGGTACCGGTGGCCTCTGCCGAGCAAGCTTTGT
GCAGTCTGGATTTACCTGGACGTGCTCTTCTCCACGGCCTCCATCATGCACCTCTGCGCCATCT
CGCTGGACCGCTACGTCGCCATCCAGAATCCCATCCACCACAGCCGCTTCAACTCCAGAACTA
AGGCATTTCTGAAAATCATTGCTGTTTGGACCATATCAGTAGGTATATCCATGCCAATACCAG
TCTTTGGGCTACAGGACGATTCGAAGGTCTTTAAGGAGGGGAGTTGCTTACTCGCCGATGATA
ACTTTGTCCTGATCGGCTCTTTTGTGTCATTTTTCATTCCCTTAACCATCATGGTGATCACCTAC
TTTCTAACTATCAAGTCACTCCAGAAAGAAGCTACTTTGTGTGTAAGTGATCTTGGCACACGG
GCCAAATTAGCTTCTTTCAGCTTCCTCCCTCAGAGTTCTTTGTCTTCAGAAAAGCTCTTCCAGC
GGTCGATCCATAGGGAGCCAGGGTCCTACACAGGCAGGAGGACTATGCAGTCCATCAGCAAT
GAGCAAAAGGCATGCAAGGTGCTGGGCATCGTCTTCTTCCTGTTTGTGGTGATGTGGTGCCCT
TTCTTCATCACAAACATCATGGCCGTCATCTGCAAAGAGTCCTGCAATGAGGATGTCATTGGG
GCCCTGCTCAATGTGTTTGTTTGGATCGGTTATCTCTCTTCAGCAGTCAACCCACTAGTCTACA
CACTGTTCAACAAGACCTATAGGTCAGCCTTTTCACGGTATATTCAGTGTCAGTACAAGGAAA
ACAAAAAACCATTGCAGTTAATTTTAGTGAACACAATACCGGCTTTGGCCTACAAGTCTAGCC
AACTTCAAATGGGACAAAAAAAGAATTCAAAGCAAGATGCCAAGACAACAGATAATGACTGC
TCAATGGTTGCTCTAGGAAAGCAGTATTCTGAAGAGGCTTCTAAAGACAATAGCGACGGAGT
GAATGAAAAGGTGAGCTGTGTGTGA

FIGURE 3B

MDILCEENTSLSSTTNSLMQLNDDNRLYSNDFNSGEANTSDAFNWTVDSENRTNLSCEGCLSPSCL
SLLHLQEKNWSALLTAVVIILTIAGNILVIMAVSLEKKLQNATNYFLMSLAIADMLLGFLVMPVSM
LTILYGYRWPLPSKLCAVWIYLDVLFSTASIMHLCAISLDRYVAIQNPIHHSRFNSRTKAFLKIIAVW
TISVGISMPIPVFGLQDDSKVFKEGSCLLADDNFVLIGSFVSFFIPLTIMVITYFLTIKSLQKEATLCVS
DLGTRAKLASFSFLPQSSLSSEKLFQRSIHREPGSYTGRRTMQSISNEQKACKVLGIVFFLFVVMWC
PFFITNIMAVICKESCNEDVIGALLNVFVWIGYLSSAVNPLVYTLFNKTYRSAFSRYIQCQYKENKK
PLQLILVNTIPALAYKSSQLQMGQKKNSKQDAKTTDNDCSMVALGKQYSEEASKDNSDGVNEKV
SCV

FIGURE 4B

MVNLRNAVHSFLVHLIGLLVWQCDISVSPVAAIVTDIFNTSDGGRFKFPDGVQNWPALSIVIIIIMTIGGN
ILVIMAVSMEKKLHNATNYFLMSLAIADMLVGLLVMPLSLLAILYDYVWPLPRYLCPVWISLDVLFSTASI
MHLCAISLDRYVAIRNPIEHSRFNSRTKAIMKIAIVWAISIGVSVPIPVIGLRDEEKVFVNNTTCVLNDPN
FVLIGSFVAFFIPLTIMVITYCLTIYVLRRQALMLLHGHTEEPPGLSLDFLKCCKRNTAEEENSANPNQDQ
NARRRKKKERRPRGTMQAINNERKASKVLGIVFFVFLIMWCPFFITNILSVLCEKSCNQKLMEKLLNVFVW
IGYVCSGINPLVYTLFNKIYRRAFSNYLRCNYKVEKKPPVRQIPRVAATALSGRELNVNIYRHTNEPVIEK
ASDNEPGIEMQVENLELPVNPSSVVSERISSV

5/19

FIGURE 4A

ATGGTGAACCTGAGGAATGCGGTGCATTCATTCCTTGTGCACCTAATTGGCCTATTGGTTTGGC
AATGTGATATTTCTGTGAGCCCAGTAGCAGCTATAGTAACTGACATTTTCAATACCTCCGATG
GTGGACGCTTCAAATTCCCAGACGGGGTACAAAACTGGCCAGCACTTTCAATCGTCATCATAA
TAATCATGACAATAGGTGGCAACATCCTTGTGATCATGGCAGTAAGCATGGAAAAGAAACTG
CACAATGCCACCAATTACTTCTTAATGTCCCTAGCCATTGCTGATATGCTAGTGGGACTACTTG
TCATGCCCCTGTCTCTCCTGGCAATCCTTTATGATTATGTCTGGCCACTACCTAGATATTTGTG
CCCCGTCTGGATTTCTTTAGATGTTTTATTTTCAACAGCGTCCATCATGCACCTCTGCGCTATAT
CGCTGGATCGGTATGTAGCAATACGTAATCCTATTGAGCATAGCCGTTTCAATTCGCGGACTA
AGGCCATCATGAAGATTGCTATTGTTTGGGCAATTTCTATAGGTGTATCAGTTCCTATCCCTGT
GATTGGACTGAGGGACGAAGAAAAGGTGTTCGTGAACAACACGACGTGCGTGCTCAACGACC
CAAATTTCGTTCTTATTGGGTCCTTCGTAGCTTTCTTCATACCGCTGACGATTATGGTGATTAC
GTATTGCCTGACCATCTACGTTCTGCGCCGACAAGCTTTGATGTTACTGCACGGCCACACCGA
GGAACCGCCTGGACTAAGTCTGGATTTCCTGAAGTGCTGCAAGAGGAATACGGCCGAGGAAG
AGAACTCTGCAAACCCTAACCAAGACCAGAACGCACGCCGAAGAAAGAAGAAGGAGAGACG
TCCTAGGGGCACCATGCAGGCTATCAACAATGAAAGAAAAGCTTCGAAAGTCCTTGGGATTG
TTTTCTTTGTGTTTCTGATCATGTGGTGCCCATTTTTCATTACCAATATTCTGTCTGTTCTTTGTG
AGAAGTCCTGTAACCAAAAGCTCATGGAAAAGCTTCTGAATGTGTTTGTTTGGATTGGCTATG
TTTGTTCAGGAATCAATCCTCTGGTGTATACTCTGTTCAACAAAATTTACCGAAGGGCATTCTC
CAACTATTTGCGTTGCAATTATAAGGTAGAGAAAAAGCCTCCTGTCAGGCAGATTCCAAGAGT
TGCCGCCACTGCTTTGTCTGGGAGGGAGCTTAATGTTAACATTTATCGGCATACCAATGAACC
GGTGATCGAGAAAGCCAGTGACAATGAGCCCGGTATAGAGATGCAAGTTGAGAATTTAGAGT
TACCAGTAAATCCCTCCAGTGTGGTTAGCGAAAGGATTAGCAGTGTGTGA

FIGURE 5A

ATGGTGAACCTGAGGAATGCGGTGCATTCATTCCTTGTGCACCTAATTGGCCTATTGGTTTGGCAAT

GTGATATTTCTGTGAGCCCAGTAGCAGCTATAGTAACTGACATTTTCAATACCTCCGATGGTGGACG

CTTCAAATTCCCAGACGGGGTACAAAACTGGCCAGCACTTTCAATCGTCATCATAATAATCATGAC

AATAGGTGGCAACATCCTTGTGATCATGGCAGTAAGCATGGAAAAGAAACTGCACAATGCCACCA

ATTACTTCTTAATGTCCCTAGCCATTGCTGATATGCTAGTGGGACTACTTGTCATGCCCCTGTCTCTC

CTGGCAATCCTTTATGATTATGTCTGGCCACTACCTAGATATTTGTGCCCCGTCTGGATTTCTTTAGA

TGTTTTATTTTCAACAGCGTCCATCATGCACCTCTGCGCTATATCGCTGGATCGGTATGTAGCAATA

CGTAATCCTATTGAGCATAGCCGTTTCAATTCGCGGACTAAGGCCATCATGAAGATTGCTATTGTTT

GGGCAATTTCTATAGGTGTATCAGTTCCTATCCCTGTGATTGGACTGAGGGACGAAGAAAAGGTGT

TCGTGAACAACACGACGTGCGTGCTCAACGACCCAAATTTCGTTCTTATTGGGTCCTTCGTAGCTTT

CTTCATACCGCTGACGATTATGGTGATTACGTATTGCCTGACCATCTACGTTCTGCGCCGACAAGCT

TTGATGTTACTGCACGGCCACACCGAGGAACCGCCTGGACTAAGTCTGGATTTCCTGAAGTGCTGC

AAGAGGAATACGGCCGAGGAAGAGAACTCTGCAAACCCTAACCAAGACCAGAACGCACGCCGAA

GAAAGAAGAAGGAGAGACGTCCTAGGGGCACCATGCAGGCTATCAACAATGAAAGAAAAGCTAA

GAAAGTCCTTGGGATTGTTTTCTTTGTGTTTCTGATCATGTGGTGCCCATTTTTCATTACCAATATTC

TGTCTGTTCTTTGTGAGAAGTCCTGTAACCAAAAGCTCATGGAAAAGCTTCTGAATGTGTTTGTTTG

GATTGGCTATGTTTGTTCAGGAATCAATCCTCTGGTGTATACTCTGTTCAACAAAATTTACCGAAGG

GCATTCTCCAACTATTTGCGTTGCAATTATAAGGTAGAGAAAAAGCCTCCTGTCAGGCAGATTCCA

AGAGTTGCCGCCACTGCTTTGTCTGGGAGGGAGCTTAATGTTAACATTTATCGGCATACCAATGAA

CCGGTGATCGAGAAAGCCAGTGACAATGAGCCCGGTATAGAGATGCAAGTTGAGAATTTAGAGTT

ACCAGTAAATCCCTCCAGTGTGGTTAGCGAAAGGATTAGCAGTGTGTGA

FIGURE 5B

MVNLRNAVHSFLVHLIGLLVWQCDISVSPVAAIVTDIFNTSDGGRFKFPDGVQNWPALSIVIIIIMTI
GGNILVIMAVSMEKKLHNATNYFLMSLAIADMLVGLLVMPLSLLAILYDYVWPLPRYLCPVWISL
DVLFSTASIMHLCAISLDRYVAIRNPIEHSRFNSRTKAIMKIAIVWAISIGVSVPIPVIGLRDEEKVFV
NNTTCVLNDPNFVLIGSFVAFFIPLTIMVITYCLTIYVLRRQALMLLHGHTEEPPGLSLDFLKCCKRN
TAEEENSANPNQDQNARRRKKKERRPRGTMQAINNERKAKKVLGIVFFVFLIMWCPFFITNILSVL
CEKSCNQKLMEKLLNVFVWIGYVCSGINPLVYTLFNKIYRRAFSNYLRCNYKVEKKPPVRQIPRV
AATALSGRELNVNIYRHTNEPVIEKASDNEPGIEMQVENLELPVNPSSVVSERISSV.

FIGURE 6B

MDILCEENTSLSSTTNSLMQLNDDNRLYSNDFNSGEANTSDAFNWTVDSENRTNLSCEGCLSPSCL
SLLHLQEKNWSALLTAVVIILTIAGNILVIMAVSLEKKLQNATNYFLMSLAIADMLLGFLVMPVSM
LTILYGYRWPLPSKLCAVWIYLDVLFSTASIMHLCAISLDRYVAIQNPIHHSRFNSRTKAFLKIIAVW
TISVGISMPIPVFGLQDDSKVFKEGSCLLADDNFVLIGSFVSFFIPLTIMVITYFLTIKVLRRQALMLL
HGHTEEPPGLSLDFLKCCKRNTAEEENSANPNQDQNARRRKKKERRPRGTMQAINNERKAS
KVLGIVFFLFVVMWCPFFITNIMAVICKESCNEDVIGALLNVFVWIGYLSSAVNPLVYTLFNKIYR
RAFSNYLRCNYKVEKKPPVRQIPRVAATALSGRELNVNIYRHTNEPVIEKASDNEPGIEMQVE
NLELPVNPSSVVSERISSV

FIGURE 6C

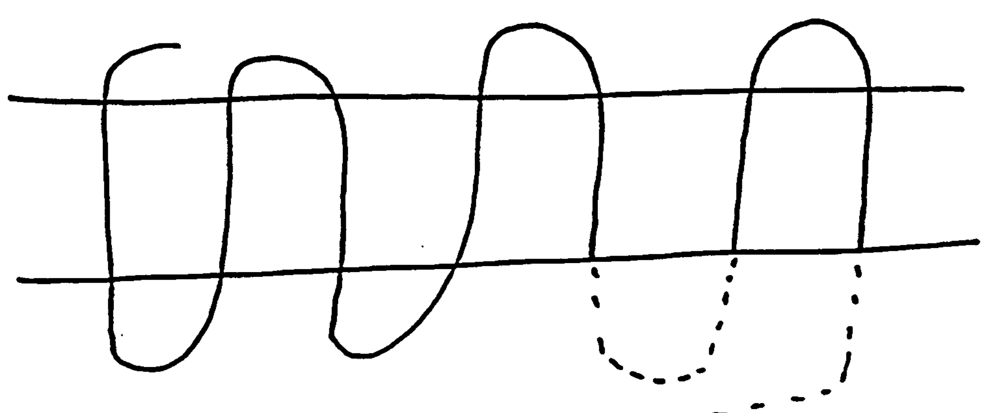


FIGURE 6A

ATGGATATTCTTTGTGAAGAAAATACTTCTTTGAGCTCAACTACGAACTCCCTAATGCAATTA
AATGATGACAACAGGCTCTACAGTAATGACTTTAACTCCGGAGAAGCTAACACTTCTGATGCA
TTTAACTGGACAGTCGACTCTGAAAATCGAACCAACCTTTCCTGTGAAGGGTGCCTCTCACCG
TCGTGTCTCTCCTTACTTCATCTCCAGGAAAAAAACTGGTCTGCTTTACTGACAGCCGTAGTGA
TTATTCTAACTATTGCTGGAAACATACTCGTCATCATGGCAGTGTCCCTAGAGAAAAAGCTGC
AGAATGCCACCAACTATTTCCTGATGTCACTTGCCATAGCTGATATGCTGCTGGGTTTCCTTGT
CATGCCCGTGTCCATGTTAACCATCCTGTATGGGTACCGGTGGCCTCTGCCGAGCAAGCTTTGT
GCAGTCTGGATTTACCTGGACGTGCTCTTCTCCACGGCCTCCATCATGCACCTCTGCGCCATCT
CGCTGGACCGCTACGTCGCCATCCAGAATCCCATCCACCACAGCCGCTTCAACTCCAGAACTA
AGGCATTTCTGAAAATCATTGCTGTTTGGACCATATCAGTAGGTATATCCATGCCAATACCAG
TCTTTGGGCTACAGGACGATTCGAAGGTCTTTAAGGAGGGGAGTTGCTTACTCGCCGATGATA
ACTTTGTCCTGATCGGCTCTTTTGTGTCATTTTTCATTCCCTTAACCATCATGGTGATCACCTAC
TTTCTAACTATCAAGGTTCTGCGCCGACAAGCTTTGATGTTACTGCACGGCCACACCGAG
GAACCGCCTGGACTAAGTCTGGATTTCCTGAAGTGCTGCAAGAGGAATACGGCCGAGGA
AGAGAACTCTGCAAACCCTAACCAAGACCAGAACGCACGCCGAAGAAAGAAGAAGGAG
AGACGTCCTAGGGGCACCATGCAGGCTATCAACAATGAAAGAAAAGCTTCGAAGGTACT
GGGCATCGTCTTCTTCCTGTTTGTGGTGATGTGGTGCCCTTTCTTCATCACAAACATCATGGCC
GTCATCTGCAAAGAGTCCTGCAATGAGGATGTCATTGGGGCCCTGCTCAATGTGTTTGTTTGG
ATCGGTTATCTCTCTTCAGCAGTCAACCCACTAGTCTATACTCTGTTCAACAAAATTTACCGA
AGGGCATTCTCCAACTATTTGCGTTGCAATTATAAGGTAGAGAAAAAGCCTCCTGTCAG
GCAGATTCCAAGAGTTGCCGCCACTGCTTTGTCTGGGAGGGAGCTTAATGTTAACATTT
ATCGGCATACCAATGAACCGGTGATCGAGAAAGCCAGTGACAATGAGCCCGGTATAGAG
ATGCAAGTTGAGAATTTAGAGTTACCAGTAAATCCCTCCAGTGTGGTTAGCGAAAGGAT
TAGCAGTGTGTGA

FIGURE 7A

ATGGATATTCTTTGTGAAGAAAATACTTCTTTGAGCTCAACTACGAACTCCCTAATGCAATTA

AATGATGACAACAGGCTCTACAGTAATGACTTTAACTCCGGAGAAGCTAACACTTCTGATGCA

TTTAACTGGACAGTCGACTCTGAAAATCGAACCAACCTTTCCTGTGAAGGGTGCCTCTCACCG

TCGTGTCTCTCCTTACTTCATCTCCAGGAAAAAAACTGGTCTGCTTTACTGACAGCCGTAGTGA

TTATTCTAACTATTGCTGGAAACATACTCGTCATCATGGCAGTGTCCCTAGAGAAAAAGCTGC

AGAATGCCACCAACTATTTCCTGATGTCACTTGCCATAGCTGATATGCTGCTGGGTTTCCTTGT

CATGCCCGTGTCCATGTTAACCATCCTGTATGGGTACCGGTGGCCTCTGCCGAGCAAGCTTTGT

GCAGTCTGGATTTACCTGGACGTGCTCTTCTCCACGGCCTCCATCATGCACCTCTGCGCCATCT

CGCTGGACCGCTACGTCGCCATCCAGAATCCCATCCACCACAGCCGCTTCAACTCCAGAACTA

AGGCATTTCTGAAAATCATTGCTGTTTGGACCATATCAGTAGGTATATCCATGCCAATACCAG

TCTTTGGGCTACAGGACGATTCGAAGGTCTTTAAGGAGGGGAGTTGCTTACTCGCCGATGATA

ACTTTGTCCTGATCGGCTCTTTTGTGTCATTTTTCATTCCCCTGACGATTATGGTGATTACGT

ATTGCCTGACCATCTACGTTCTGCGCCGACAAGCTTTGATGTTACTGCACGGCCACACC

GAGGAACCGCCTGGACTAAGTCTGGATTTCCTGAAGTGCTGCAAGAGGAATACGGCCGA

GGAAGAGAACTCTGCAAACCCTAACCAAGACCAGAACGCACGCCGAAGAAAGAAGAAG

GAGAGACGTCCTAGGGGCACCATGCAGGCTATCAACAATGAAAGAAAAGCTAAGAAAGT

CCTTGGGATTGTTTTCTTTGTGTTTCTGATCATGTGGTGCCCTTTCTTCATCACAAACATCA

TGGCCGTCATCTGCAAAGAGTCCTGCAATGAGGATGTCATTGGGGCCCTGCTCAATGTGTTTG

TTTGGATCGGTTATCTCTCTTCAGCAGTCAACCCACTAGTCTATACTCTGTTCAACAAAATTT

ACCGAAGGGCATTCTCCAACTATTTGCGTTGCAATTATAAGGTAGAGAAAAAGCCTCCT

GTCAGGCAGATTCCAAGAGTTGCCGCCACTGCTTTGTCTGGGAGGGAGCTTAATGTTAA

CATTTATCGGCATACCAATGAACCGGTGATCGAGAAAGCCAGTGACAATGAGCCCGGTA

TAGAGATGCAAGTTGAGAATTTAGAGTTACCAGTAAATCCCTCCAGTGTGGTTAGCGAA

AGGATTAGCAGTGTGTGA

FIGURE 7B

MDILCEENTSLSSTTNSLMQLNDDNRLYSNDFNSGEANTSDAFNWTVDSENRTNLSCEGCLSPSCL
SLLHLQEKNWSALLTAVVIILTIAGNILVIMAVSLEKKLQNATNYFLMSLAIADMLLGFLVMPVSM
LTILYGYRWPLPSKLCAVWIYLDVLFSTASIMHLCAISLDRYVAIQNPIHHSRFNSRTKAFLKIIAVW
TISVGISMPIPVFGLQDDSKVFKEGSCLLADDNFVLIGSFVSFFIPLTIMVITYCLTIIYVLRRQALML
LHGHTEEPPGLSLDFLKCCKRNTAEEENSANPNQDQNARRRKKKERRPRGTMQAINNERKA
KKVLGIVFFVFLIMWCPFFITNIMAVICKESCNEDVIGALLNVFVWIGYLSSAVNPLVYTLFNKTY
RRAFSNYLRCNYKVEKKPPVRQIPRVAATALSGRELNVNIYRHTNEPVIEKASDNEPGIEMQV
ENLELPVNPSSVVSERISSV

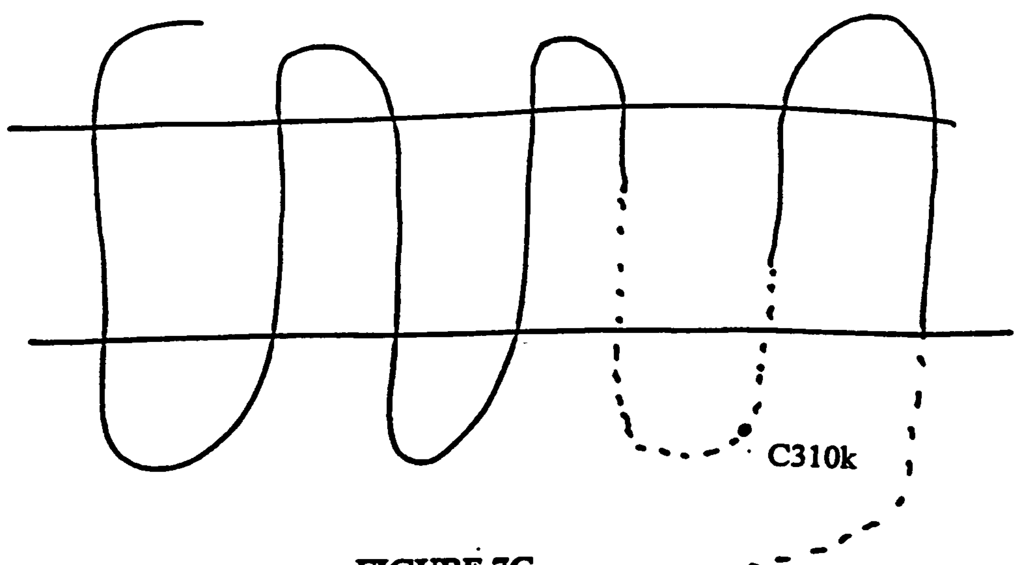


FIGURE 7C

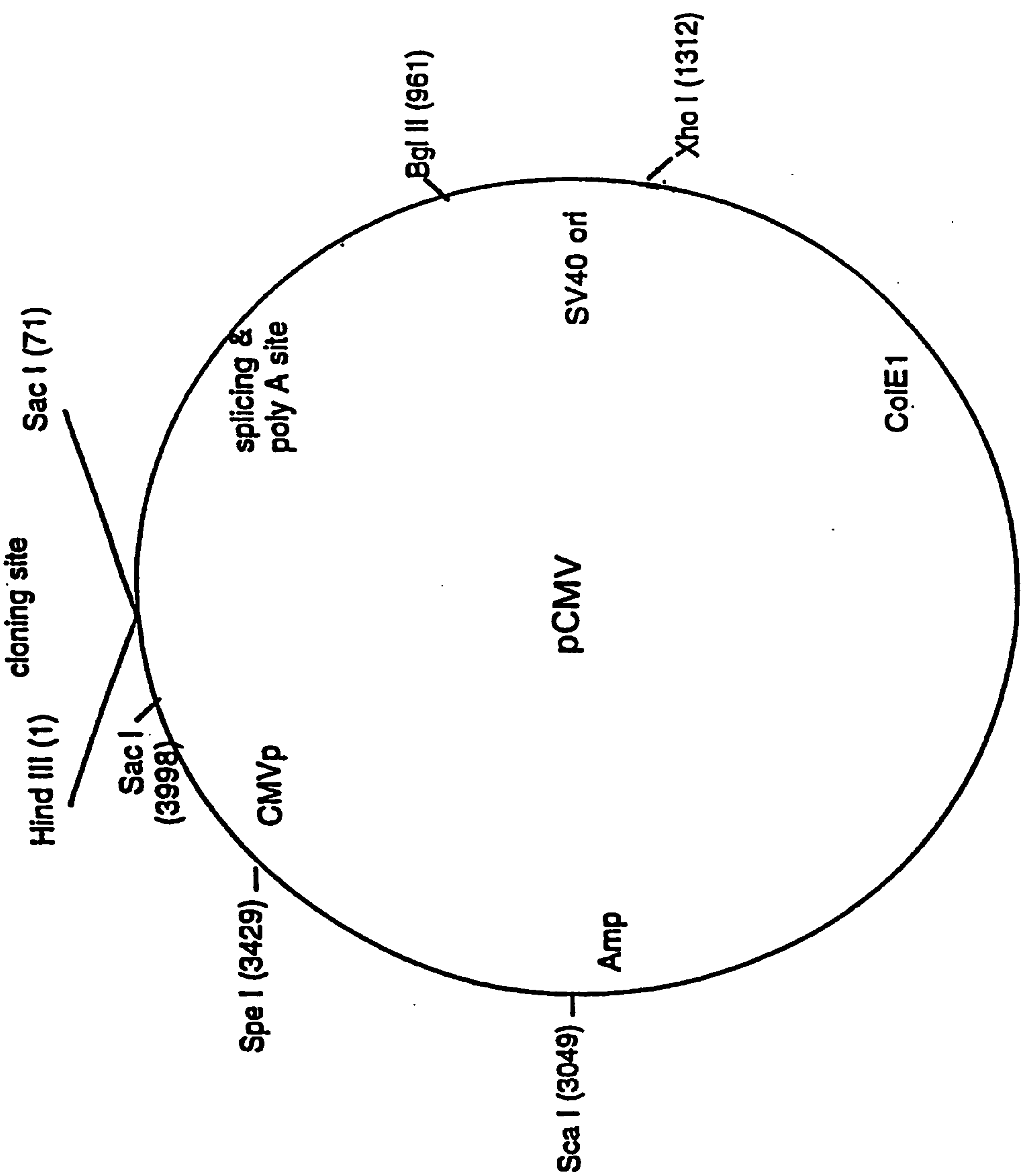

Xho I (1312)
Bgl II (961)
SV40 ori
splicing &
poly A site
Sac I (71)
ColE1
cloning site
pCMV
Hind III (1)
Sac I
(3998)
CMVp
Spe I (3429)
Amp
Sca I (3049)

FIGURE 8

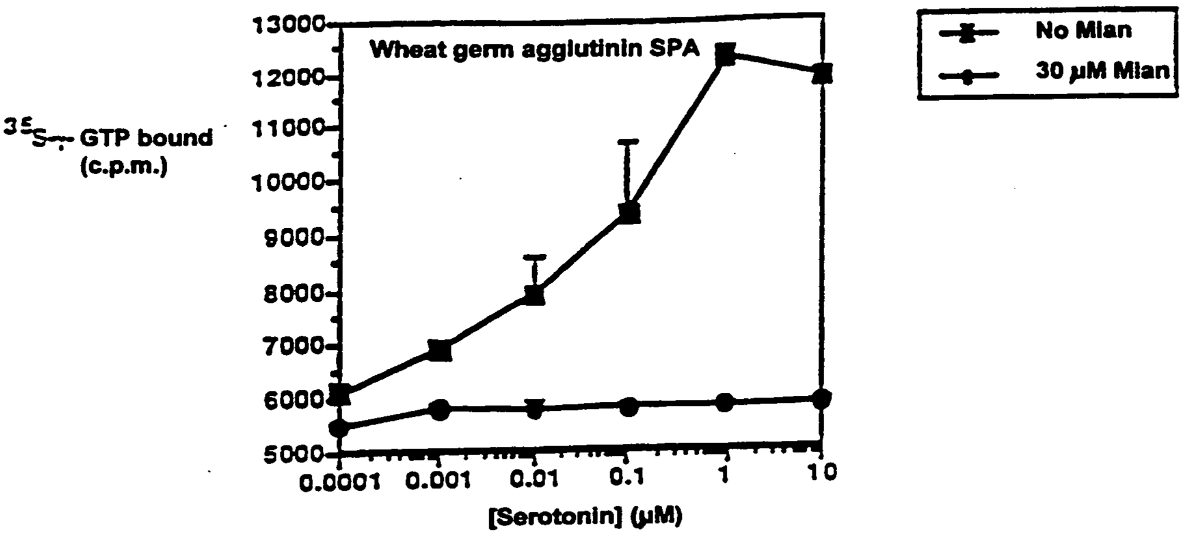


FIGURE 9

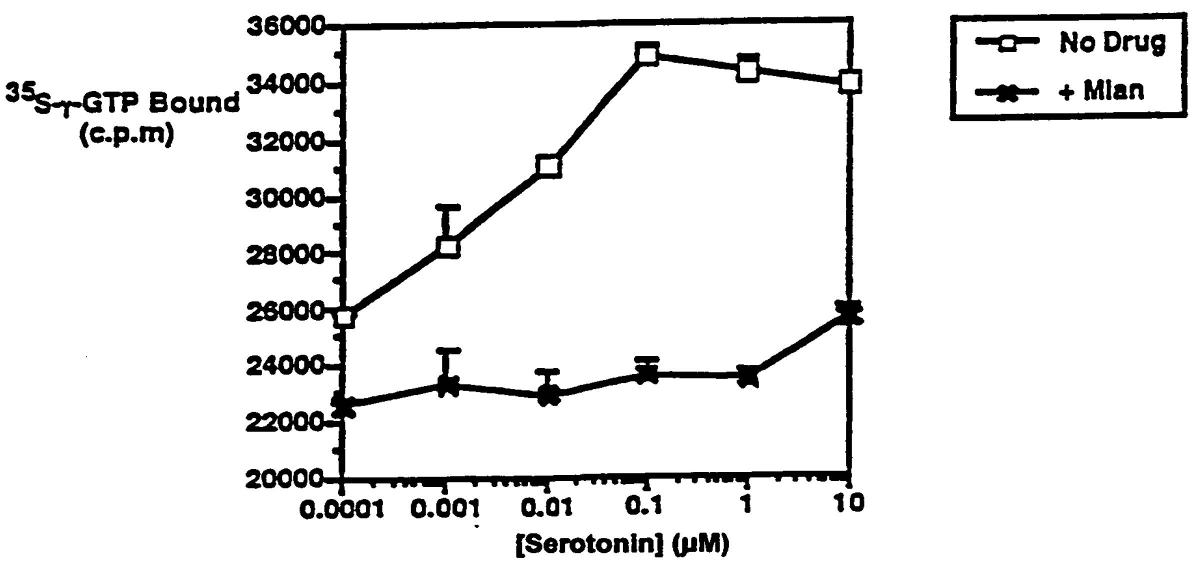
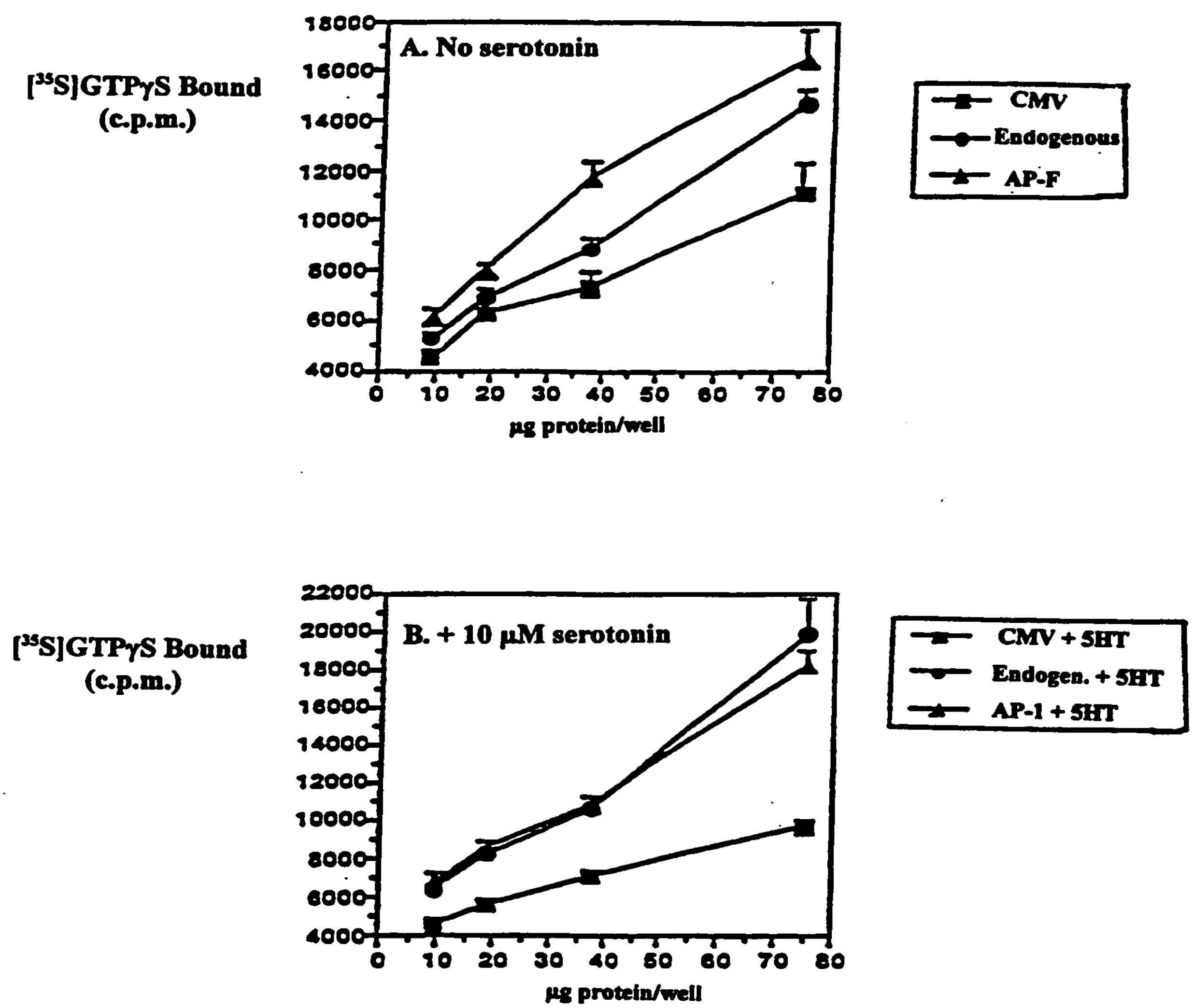


FIGURE 10



A. No serotonin
[35S]GTPγS Bound (c.p.m.)
18000
16000
14000
12000
10000
8000
6000
4000
0 10 20 30 40 50 60 70 80
μg protein/well
CMV
Endogenous
AP-F
B. + 10 μM serotonin
[35S]GTPγS Bound (c.p.m.)
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
0 10 20 30 40 50 60 70 80
μg protein/well
CMV + 5HT
Endogen. + 5HT
AP-1 + 5HT

FIGURE 11

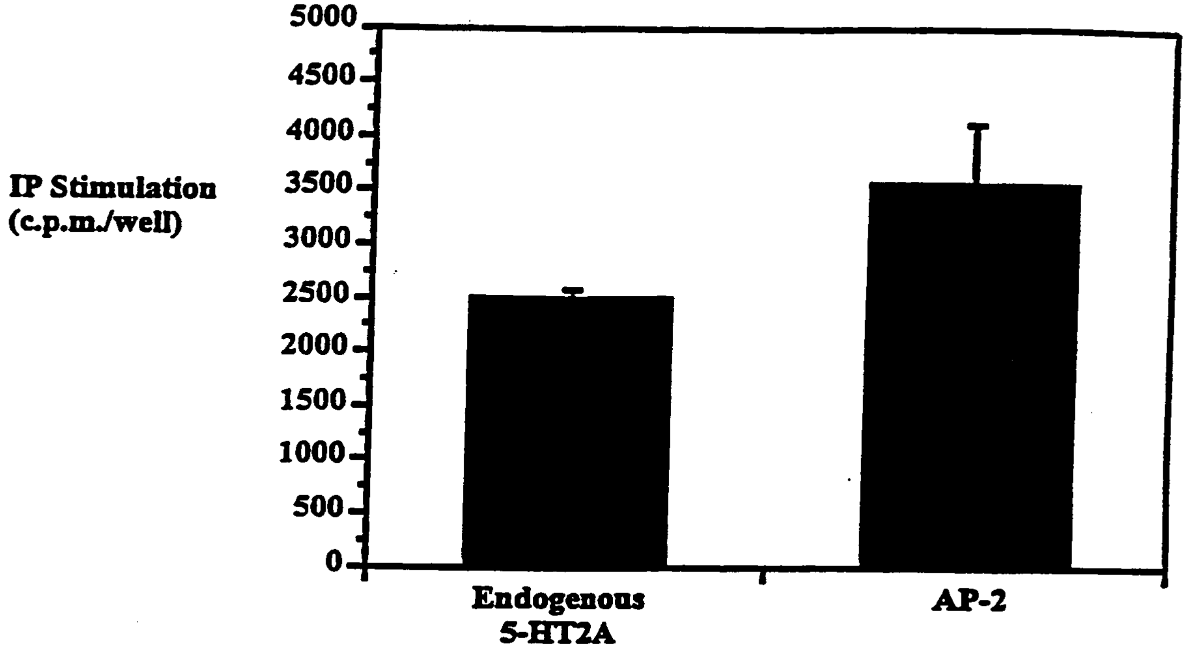
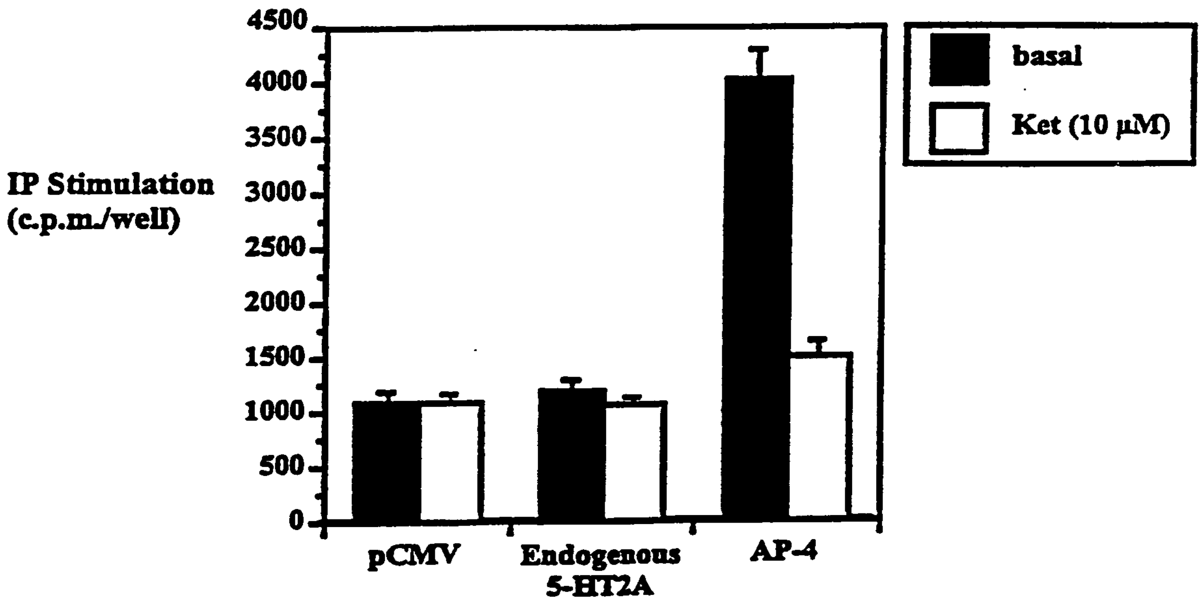

IP Stimulation
(c.p.m./well)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Endogenous
5-HT2A
AP-2

FIGURE 12



IP Stimulation
(c.p.m./well)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
basal
Ket (10 µM)
pCMV
Endogenous
5-HT2A
AP-4

FIGURE 13

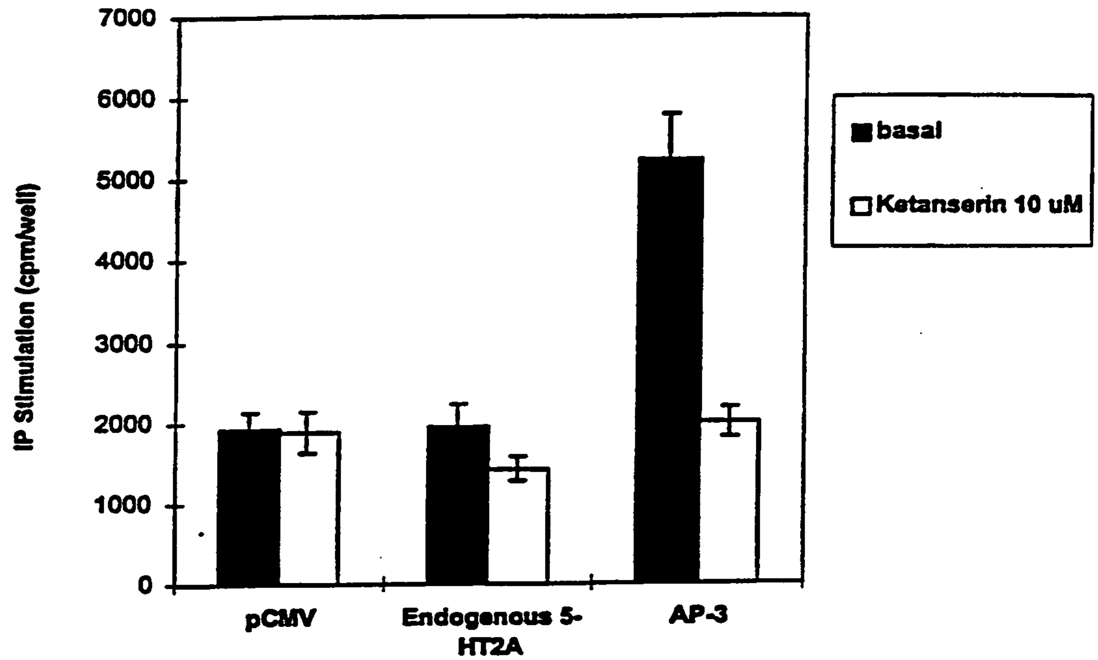

7000
6000
5000
4000
3000
2000
1000
0
IP Stimulation (cpm/well)
basal
Ketanserin 10 uM
pCMV
Endogenous 5-HT2A
AP-3

FIGURE 14

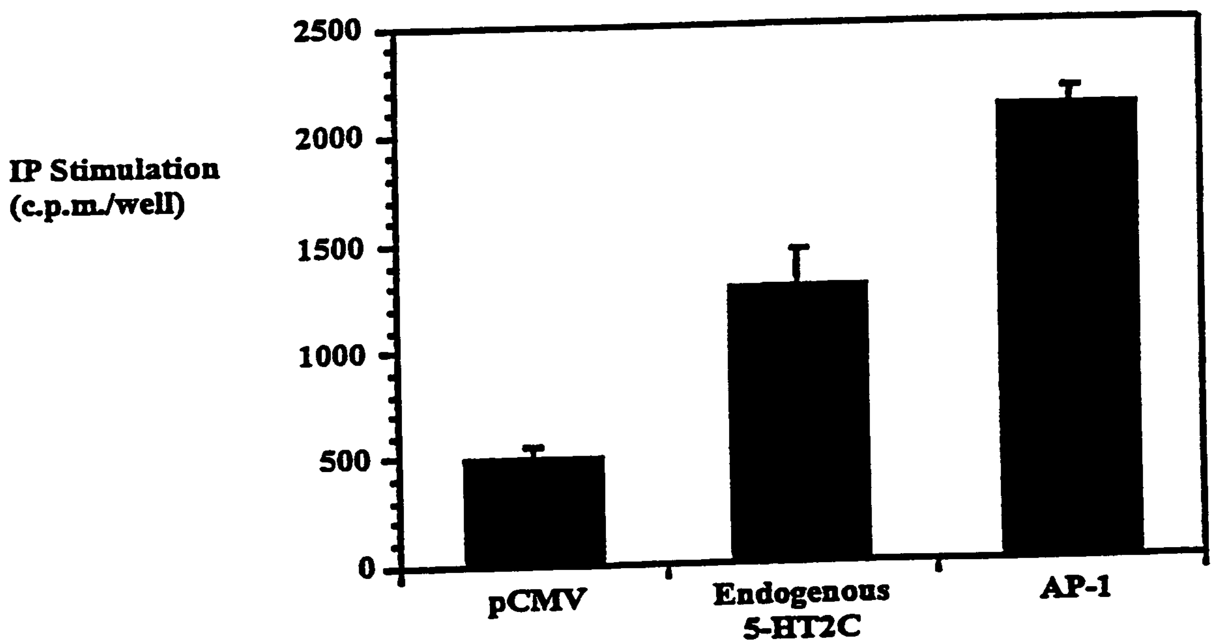

IP Stimulation
(c.p.m./well)
2500
2000
1500
1000
500
0
pCMV
Endogenous
5-HT2C
AP-1

FIGURE 15

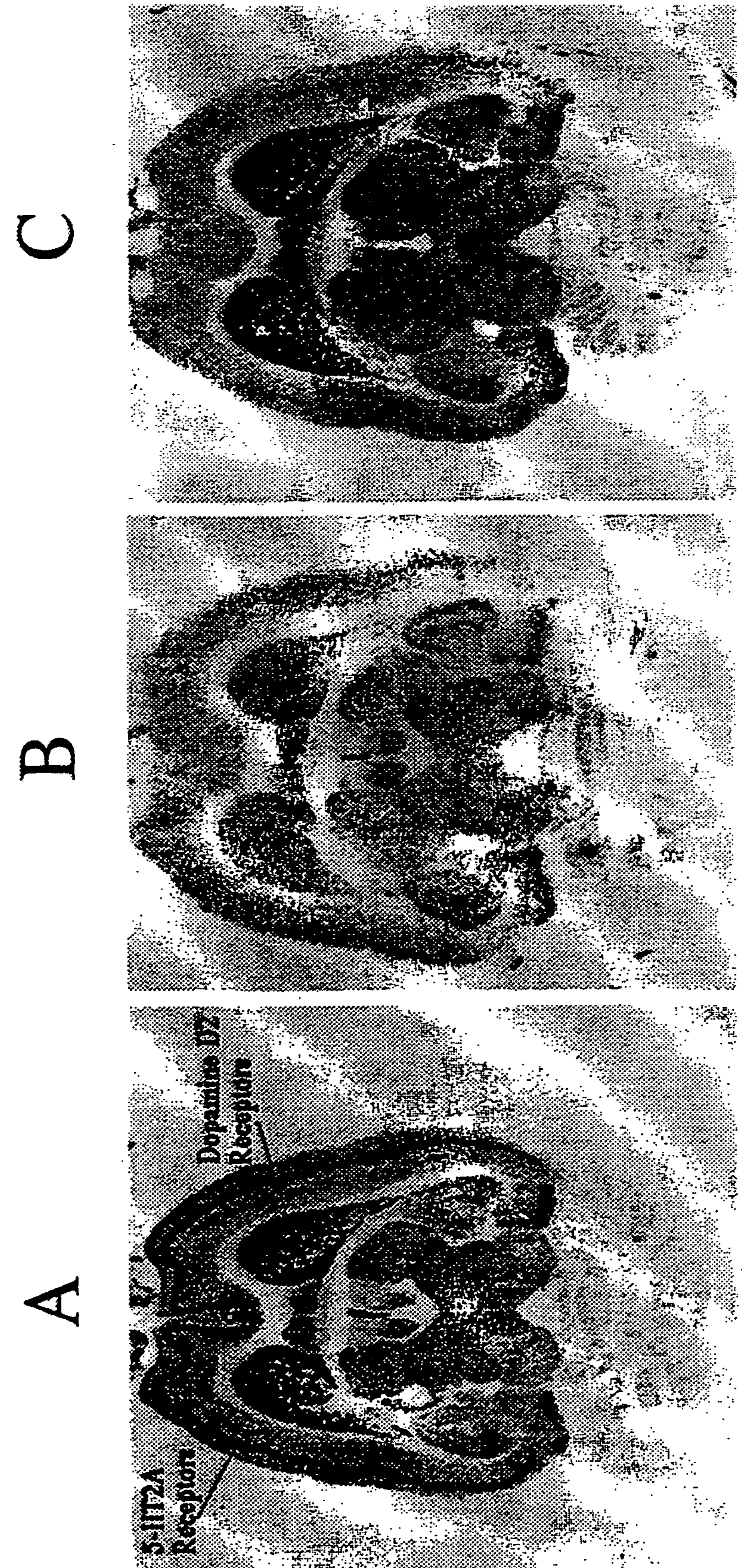


Figure 16

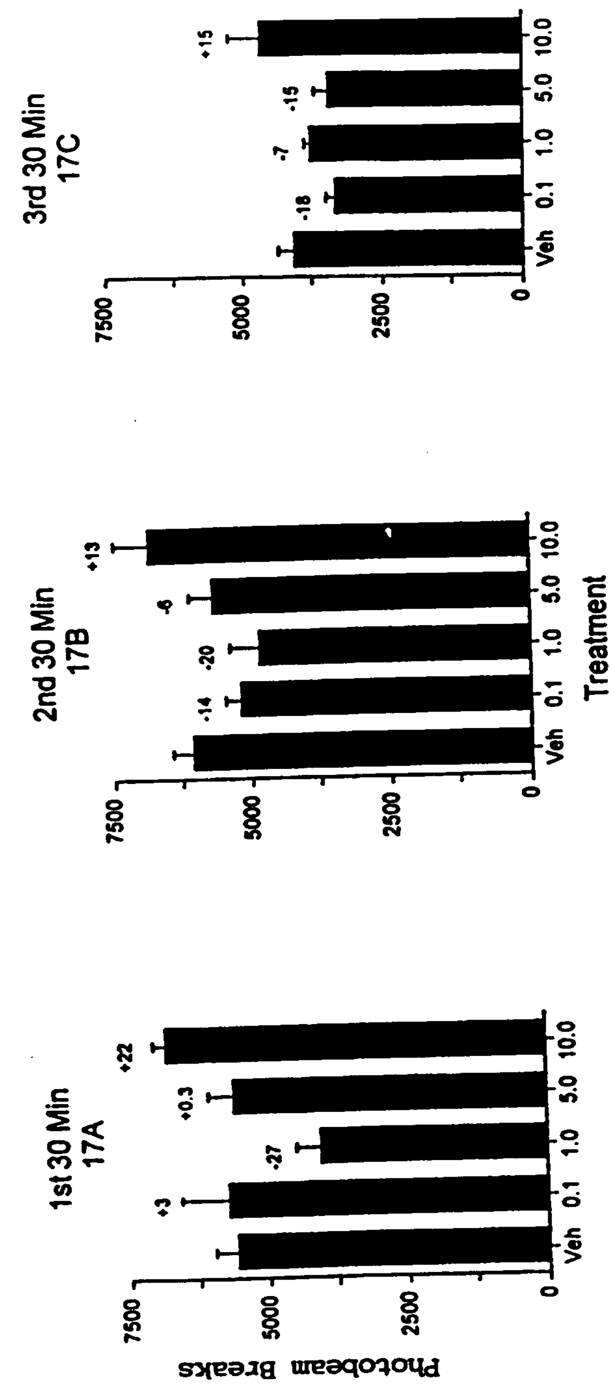

3rd 30 Min
17C
2nd 30 Min
17B
1st 30 Min
17A
Photobeam Breaks
Treatment
7500
5000
2500
0
Veh
0.1
1.0
5.0
10.0
+15
-15
-7
-18
+13
-6
-20
-14
+22
+0.3
-27
+3

Figure 17

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: Arena Pharmaceuticals, Inc. and Tripos, Inc.

(ii) TITLE OF INVENTION: Non-Endogenous, Constitutively Activated Human Serotonin Receptors and Small Molecule Modulators Thereof

(iii) NUMBER OF SEQUENCES: 33

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Woodcock Washburn Kurtz Mackiewicz & Norris LLP
(B) STREET: One Liberty Place - 46th Floor
(C) CITY: Philadelphia
(D) STATE: PA
(E) COUNTRY: USA
(F) ZIP: 19103

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: WINDOWS NT, Version #4.0
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US99/08168
(B) FILING DATE: April 14, 1999
(C) CLASSIFICATION: 435

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Mark J. Rosen
(B) REGISTRATION NUMBER: 39,822

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (215) 568-3100
(B) TELEFAX: (215) 568-3439

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GACCTCGAGG TTGCTTAAGA CTGAAGC 27

(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATTTCTAGAC ATATGTAGCT TGTACCG 27

(4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTAGGGGCAC CATGCAGGCT ATCAACAATG AAAGAAAAGC TAAGAAAGTC 50

(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CAAGGACTTT CTTAGCTTTT CTTTCATTGT TGATAGCCTG CATGGTGCCC 50

(6) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GACCTCGAGT CCTTCTACAC CTCATC 26

(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TGCTCTAGAT TCCAGATAGG TGAAAACTTG 30

(8) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAAAGAAAGT ACTGGGCATC GTCTTCTTCC T 31

(9) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCGCTCGAGT ACTGCGCCGA CAAGCTTTGA T 31

(10) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGATGCCCAG CACTTTCGAA GCTTTTCTTT CATTGTTG 38

(11) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AAAAGCTTCG AAAGTGCTGG GCATCGTCTT CTTCCT 36

(12) INFORMATION FOR SEQ ID NO:11

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGCTCTAGAT TCCAGATAGG TGAAAACTTG 30

(13) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGTGTCTCTC CTTACTTCA 19

(14) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TCGGCGCAGT ACTTTGATAG TTAGAAAGTA GGTGAT 36

(15) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TTCTAACTAT CAAAGTACTG CGCCGACAAG CTTTGATG 38

(16) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TTCAGCAGTC AACCCACTAG TCTATACTCT GTTCAACAAA ATT 43

(17) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ATTTCTAGAC ATATGTAGCT TGTACCGT 28

(18) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATCACCTACT TTCTAACTA 19

(19) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CCATAATCGT CAGGGGAATG AAAAATGACA CAA 33

(20) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATTTTTCATT CCCCTGACGA TTATGGTGAT TAC 33

(21) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TGATGAAGAA AGGGCACCAC ATGATCAGAA ACA 33

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GATCATGTGG TGCCCTTTCT TCATCACAAA CAT 33

(23) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GAGACATATT ATCTGCCACG GAGG 24

(24) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TTGGCATAGA AACCGGACCC AAGG 24

(25) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1416 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATGGATATTC TTTGTGAAGA AAATACTTCT TTGAGCTCAA CTACGAACTC CCTAATGCAA 60

TTAAATGATG ACAACAGGCT CTACAGTAAT GACTTTAACT CCGGAGAAGC TAACACTTCT 120

GATGCATTTA ACTGGACAGT CGACTCTGAA AATCGAACCA ACCTTTCCTG TGAAGGGTGC 180

CTCTCACCGT CGTGTCTCTC CTTACTTCAT CTCCAGGAAA AAAACTGGTC TGCTTTACTG 240

ACAGCCGTAG TGATTATTCT AACTATTGCT GGAAACATAC TCGTCATCAT GGCAGTGTCC 300

CTAGAGAAAA AGCTGCAGAA TGCCACCAAC TATTTCCTGA TGTCACTTGC CATAGCTGAT 360

ATGCTGCTGG GTTTCCTTGT CATGCCCGTG TCCATGTTAA CCATCCTGTA TGGGTACCGG 420

TGGCCTCTGC CGAGCAAGCT TTGTGCAGTC TGGATTTACC TGGACGTGCT CTTCTCCACG 480

GCCTCCATCA TGCACCTCTG CGCCATCTCG CTGGACCGCT ACGTCGCCAT CCAGAATCCC 540

ATCCACCACA GCCGCTTCAA CTCCAGAACT AAGGCATTTC TGAAAATCAT TGCTGTTTGG 600

ACCATATCAG TAGGTATATC CATGCCAATA CCAGTCTTTG GGCTACAGGA CGATTCGAAG 660

GTCTTTAAGG AGGGGAGTTG CTTACTCGCC GATGATAACT TTGTCCTGAT CGGCTCTTTT 720

GTGTCATTTT TCATTCCCTT AACCATCATG GTGATCACCT ACTTTCTAAC TATCAAGTCA 780

CTCCAGAAAG AAGCTACTTT GTGTGTAAGT GATCTTGGCA CACGGGCCAA ATTAGCTTCT 840

TTCAGCTTCC TCCCTCAGAG TTCTTTGTCT TCAGAAAAGC TCTTCCAGCG GTCGATCCAT 900

AGGGAGCCAG GGTCCTACAC AGGCAGGAGG ACTATGCAGT CCATCAGCAA TGAGCAAAAG 960

GCATGCAAGG TGCTGGGCAT CGTCTTCTTC CTGTTTGTGG TGATGTGGTG CCCTTTCTTC 1020

ATCACAAACA TCATGGCCGT CATCTGCAAA GAGTCCTGCA ATGAGGATGT CATTGGGGCC 1080

CTGCTCAATG TGTTTGTTTG GATCGGTTAT CTCTCTTCAG CAGTCAACCC ACTAGTCTAC 1140

ACACTGTTCA ACAAGACCTA TAGGTCAGCC TTTTCACGGT ATATTCAGTG TCAGTACAAG 1200

GAAAACAAAA AACCATTGCA GTTAATTTTA GTGAACACAA TACCGGCTTT GGCCTACAAG 1260

TCTAGCCAAC TTCAAATGGG ACAAAAAAAG AATTCAAAGC AAGATGCCAA GACAACAGAT 1320

AATGACTGCT CAATGGTTGC TCTAGGAAAG CAGTATTCTG AAGAGGCTTC TAAAGACAAT 1380

AGCGACGGAG TGAATGAAAA GGTGAGCTGT GTGTGA 1416

(26) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 470 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Met Asp Ile Leu Cys Glu Glu Asn Thr Ser Leu Ser Ser Thr Thr Asn
1 5 10 15

Ser Leu Met Gln Leu Asn Asp Asp Asn Arg Leu Tyr Ser Asn Asp Phe
20 25 30

Asn Ser Gly Glu Ala Asn Thr Ser Asp Ala Phe Asn Trp Thr Val Asp
35 40 45

Ser Glu Asn Arg Thr Asn Leu Ser Cys Glu Gly Cys Leu Ser Pro Ser
50 55 60

Cys Ser Leu Leu His Leu Gln Glu Lys Asn Trp Ser Ala Leu Leu Thr
65 70 75 80

Ala Val Val Ile Ile Leu Thr Ile Ala Gly Asn Ile Leu Val Ile Met
85 90 95

Ala Val Ser Leu Glu Lys Lys Leu Gln Asn Ala Thr Asn Tyr Phe Leu
100 105 110

Met Ser Leu Ala Ile Ala Asp Met Leu Leu Gly Phe Leu Val Met Pro
115 120 125

Val Ser Met Leu Thr Ile Leu Tyr Gly Tyr Arg Trp Pro Leu Pro Ser
130 135 140

Lys Leu Cys Ala Val Trp Ile Tyr Leu Asp Val Leu Phe Ser Thr Ala
145 150 155 160

```
Ser Ile Met His Leu Cys Ala Ile Ser Leu Asp Arg Tyr Val Ala Ile
                165                 170                 175

Gln Asn Pro Ile His His Ser Arg Phe Asn Ser Arg Thr Lys Ala Phe
            180                 185                 190

Leu Lys Ile Ile Ala Val Trp Thr Ile Ser Val Gly Ile Ser Met Pro
        195                 200                 205

Ile Pro Val Phe Gly Leu Gln Asp Asp Ser Lys Val Phe Lys Glu Gly
    210                 215                 220

Ser Cys Leu Leu Ala Asp Asp Asn Phe Val Leu Ile Gly Ser Phe Val
225                 230                 235                 240

Ser Phe Phe Ile Pro Leu Thr Ile Met Val Ile Thr Tyr Phe Leu Thr
                245                 250                 255

Ile Lys Ser Leu Gln Lys Glu Ala Thr Leu Cys Val Ser Asp Leu Gly
            260                 265                 270

Thr Arg Ala Lys Leu Ala Ser Phe Ser Phe Leu Pro Gln Ser Ser Leu
        275                 280                 285

Ser Ser Glu Lys Leu Phe Gln Arg Ser Ile His Arg Glu Pro Gly Ser
    290                 295                 300

Tyr Thr Gly Arg Arg Thr Met Gln Ser Ile Ser Asn Glu Gln Lys Ala
305                 310                 315                 320

Cys Lys Val Leu Gly Ile Val Phe Phe Leu Phe Val Val Met Trp Cys
                325                 330                 335

Pro Phe Phe Ile Thr Asn Ile Met Ala Val Ile Cys Lys Glu Ser Cys
            340                 345                 350

Asn Glu Asp Val Ile Gly Ala Leu Leu Asn Val Phe Val Trp Ile Gly
        355                 360                 365

Tyr Leu Ser Ser Ala Val Asn Pro Leu Val Tyr Thr Leu Phe Asn Lys
    370                 375                 380

Thr Tyr Arg Ser Ala Phe Ser Arg Tyr Ile Gln Cys Gln Tyr Lys Glu
385                 390                 395                 400

Asn Lys Lys Pro Leu Gln Leu Ile Leu Val Asn Thr Ile Pro Ala Leu
                405                 410                 415

Ala Tyr Lys Ser Ser Gln Leu Gln Met Gly Gln Lys Lys Asn Ser Lys
            420                 425                 430

Gln Asp Ala Lys Thr Thr Asp Asn Asp Cys Ser Met Val Ala Leu Gly
        435                 440                 445

Lys Gln Tyr Ser Glu Glu Ala Ser Lys Asp Asn Ser Asp Gly Val Asn
    450                 455                 460

Glu Lys Val Ser Cys Val
465                 470
```

(27) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1377 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ATGGTGAACC TGAGGAATGC GGTGCATTCA TTCCTTGTGC ACCTAATTGG CCTATTGGTT 60
TGGCAATGTG ATATTTCTGT GAGCCCAGTA GCAGCTATAG TAACTGACAT TTTCAATACC 120
TCCGATGGTG GACGCTTCAA ATTCCCAGAC GGGGTACAAA ACTGGCCAGC ACTTTCAATC 180
GTCATCATAA TAATCATGAC AATAGGTGGC AACATCCTTG TGATCATGGC AGTAAGCATG 240
GAAAAGAAAC TGCACAATGC CACCAATTAC TTCTTAATGT CCCTAGCCAT TGCTGATATG 300
CTAGTGGGAC TACTTGTCAT GCCCCTGTCT CTCCTGGCAA TCCTTTATGA TTATGTCTGG 360
CCACTACCTA GATATTTGTG CCCCGTCTGG ATTTCTTTAG ATGTTTTATT TTCAACAGCG 420
TCCATCATGC ACCTCTGCGC TATATCGCTG GATCGGTATG TAGCAATACG TAATCCTATT 480
GAGCATAGCC GTTTCAATTC GCGGACTAAG GCCATCATGA AGATTGCTAT TGTTTGGGCA 540
ATTTCTATAG GTGTATCAGT TCCTATCCCT GTGATTGGAC TGAGGGACGA AGAAAAGGTG 600
TTCGTGAACA ACACGACGTG CGTGCTCAAC GACCCAAATT TCGTTCTTAT TGGGTCCTTC 660
GTAGCTTTCT TCATACCGCT GACGATTATG GTGATTACGT ATTGCCTGAC CATCTACGTT 720
CTGCGCCGAC AAGCTTTGAT GTTACTGCAC GGCCACACCG AGGAACCGCC TGGACTAAGT 780
CTGGATTTCC TGAAGTGCTG CAAGAGGAAT ACGGCCGAGG AAGAGAACTC TGCAAACCCT 840
AACCAAGACC AGAACGCACG CCGAAGAAAG AAGAAGGAGA GACGTCCTAG GGGCACCATG 900
CAGGCTATCA ACAATGAAAG AAAAGCTTCG AAAGTCCTTG GGATTGTTTT CTTTGTGTTT 960
CTGATCATGT GGTGCCCATT TTTCATTACC AATATTCTGT CTGTTCTTTG TGAGAAGTCC 1020
TGTAACCAAA AGCTCATGGA AAAGCTTCTG AATGTGTTTG TTTGGATTGG CTATGTTTGT 1080
TCAGGAATCA ATCCTCTGGT GTATACTCTG TTCAACAAAA TTTACCGAAG GGCATTCTCC 1140
AACTATTTGC GTTGCAATTA TAAGGTAGAG AAAAAGCCTC CTGTCAGGCA GATTCCAAGA 1200
GTTGCCGCCA CTGCTTTGTC TGGGAGGGAG CTTAATGTTA ACATTTATCG GCATACCAAT 1260
GAACCGGTGA TCGAGAAAGC CAGTGACAAT GAGCCCGGTA TAGAGATGCA AGTTGAGAAT 1320
TTAGAGTTAC CAGTAAATCC CTCCAGTGTG GTTAGCGAAA GGATTAGCAG TGTGTGA 1377

(28) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 458 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Met Val Asn Leu Arg Asn Ala Val His Ser Phe Leu Val His Leu Ile
1               5                   10                  15

Gly Leu Leu Val Trp Gln Cys Asp Ile Ser Val Ser Pro Val Ala Ala
            20                  25                  30

Ile Val Thr Asp Ile Phe Asn Thr Ser Asp Gly Gly Arg Phe Lys Phe
        35                  40                  45

Pro Asp Gly Val Gln Asn Trp Pro Ala Leu Ser Ile Val Ile Ile Ile
    50                  55                  60

Ile Met Thr Ile Gly Gly Asn Ile Leu Val Ile Met Ala Val Ser Met
65                  70                  75                  80

Glu Lys Lys Leu His Asn Ala Thr Asn Tyr Phe Leu Met Ser Leu Ala
                85                  90                  95

Ile Ala Asp Met Leu Val Gly Leu Leu Val Met Pro Leu Ser Leu Leu
            100                 105                 110

Ala Ile Leu Tyr Asp Tyr Val Trp Pro Leu Pro Arg Tyr Leu Cys Pro
        115                 120                 125

Val Trp Ile Ser Leu Asp Val Leu Phe Ser Thr Ala Ser Ile Met His
    130                 135                 140

Leu Cys Ala Ile Ser Leu Asp Arg Tyr Val Ala Ile Arg Asn Pro Ile
145                 150                 155                 160

Glu His Ser Arg Phe Asn Ser Arg Thr Lys Ala Ile Met Lys Ile Ala
                165                 170                 175

Ile Val Trp Ala Ile Ser Ile Gly Val Ser Val Pro Ile Pro Val Ile
            180                 185                 190

Gly Leu Arg Asp Glu Glu Lys Val Phe Val Asn Asn Thr Thr Cys Val
        195                 200                 205

Leu Asn Asp Pro Asn Phe Val Leu Ile Gly Ser Phe Val Ala Phe Phe
    210                 215                 220

Ile Pro Leu Thr Ile Met Val Ile Thr Tyr Cys Leu Thr Ile Tyr Val
225                 230                 235                 240

Leu Arg Arg Gln Ala Leu Met Leu Leu His Gly His Thr Glu Glu Pro
                245                 250                 255

Pro Gly Leu Ser Leu Asp Phe Leu Lys Cys Cys Lys Arg Asn Thr Ala
            260                 265                 270
```

```
Glu Glu Glu Asn Ser Ala Asn Pro Asn Gln Asp Gln Asn Ala Arg Arg
        275                 280                 285

Arg Lys Lys Lys Glu Arg Arg Pro Arg Gly Thr Met Gln Ala Ile Asn
    290                 295                 300

Asn Glu Arg Lys Ala Ser Lys Val Leu Gly Ile Val Phe Phe Val Phe
305                 310                 315                 320

Leu Ile Met Trp Cys Pro Phe Phe Ile Thr Asn Ile Leu Ser Val Leu
                325                 330                 335

Cys Glu Lys Ser Cys Asn Gln Lys Leu Met Glu Lys Leu Leu Asn Val
            340                 345                 350

Phe Val Trp Ile Gly Tyr Val Cys Ser Gly Ile Asn Pro Leu Val Tyr
        355                 360                 365

Thr Leu Phe Asn Lys Ile Tyr Arg Arg Ala Phe Ser Asn Tyr Leu Arg
    370                 375                 380

Cys Asn Tyr Lys Val Glu Lys Lys Pro Pro Val Arg Gln Ile Pro Arg
385                 390                 395                 400

Val Ala Ala Thr Ala Leu Ser Gly Arg Glu Leu Asn Val Asn Ile Tyr
                405                 410                 415

Arg His Thr Asn Glu Pro Val Ile Glu Lys Ala Ser Asp Asn Glu Pro
            420                 425                 430

Gly Ile Glu Met Gln Val Glu Asn Leu Glu Leu Pro Val Asn Pro Ser
        435                 440                 445

Ser Val Val Ser Glu Arg Ile Ser Ser Val
    450                 455
```

(29) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1377 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
ATGGTGAACC TGAGGAATGC GGTGCATTCA TTCCTTGTGC ACCTAATTGG CCTATTGGTT     60
TGGCAATGTG ATATTTCTGT GAGCCCAGTA GCAGCTATAG TAACTGACAT TTTCAATACC    120
TCCGATGGTG GACGCTTCAA ATTCCCAGAC GGGGTACAAA ACTGGCCAGC ACTTTCAATC    180
GTCATCATAA TAATCATGAC AATAGGTGGC AACATCCTTG TGATCATGGC AGTAAGCATG    240
GAAAAGAAAC TGCACAATGC CACCAATTAC TTCTTAATGT CCCTAGCCAT TGCTGATATG    300
CTAGTGGGAC TACTTGTCAT GCCCCTGTCT CTCCTGGCAA TCCTTTATGA TTATGTCTGG    360
```

```
CCACTACCTA GATATTTGTG CCCCGTCTGG ATTTCTTTAG ATGTTTTATT TTCAACAGCG    420
TCCATCATGC ACCTCTGCGC TATATCGCTG GATCGGTATG TAGCAATACG TAATCCTATT    480
GAGCATAGCC GTTTCAATTC GCGGACTAAG GCCATCATGA AGATTGCTAT TGTTTGGGCA    540
ATTTCTATAG GTGTATCAGT TCCTATCCCT GTGATTGGAC TGAGGGACGA AGAAAAGGTG    600
TTCGTGAACA ACACGACGTG CGTGCTCAAC GACCCAAATT TCGTTCTTAT TGGGTCCTTC    660
GTAGCTTTCT TCATACCGCT GACGATTATG GTGATTACGT ATTGCCTGAC CATCTACGTT    720
CTGCGCCGAC AAGCTTTGAT GTTACTGCAC GGCCACACCG AGGAACCGCC TGGACTAAGT    780
CTGGATTTCC TGAAGTGCTG CAAGAGGAAT ACGGCCGAGG AAGAGAACTC TGCAAACCCT    840
AACCAAGACC AGAACGCACG CCGAAGAAAG AAGAAGGAGA GACGTCCTAG GGGCACCATG    900
CAGGCTATCA ACAATGAAAG AAAAGCTAAG AAAGTCCTTG GGATTGTTTT CTTTGTGTTT    960
CTGATCATGT GGTGCCCATT TTTCATTACC AATATTCTGT CTGTTCTTTG TGAGAAGTCC   1020
TGTAACCAAA AGCTCATGGA AAAGCTTCTG AATGTGTTTG TTTGGATTGG CTATGTTTGT   1080
TCAGGAATCA ATCCTCTGGT GTATACTCTG TTCAACAAAA TTTACCGAAG GGCATTCTCC   1140
AACTATTTGC GTTGCAATTA TAAGGTAGAG AAAAAGCCTC CTGTCAGGCA GATTCCAAGA   1200
GTTGCCGCCA CTGCTTTGTC TGGGAGGGAG CTTAATGTTA ACATTTATCG GCATACCAAT   1260
GAACCGGTGA TCGAGAAAGC CAGTGACAAT GAGCCCGGTA TAGAGATGCA AGTTGAGAAT   1320
TTAGAGTTAC CAGTAAATCC CTCCAGTGTG GTTAGCGAAA GGATTAGCAG TGTGTGA      1377
```

(30) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 458 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Met Val Asn Leu Arg Asn Ala Val His Ser Phe Leu Val His Leu Ile
1               5                   10                  15

Gly Leu Leu Val Trp Gln Cys Asp Ile Ser Val Ser Pro Val Ala Ala
            20                  25                  30

Ile Val Thr Asp Ile Phe Asn Thr Ser Asp Gly Gly Arg Phe Lys Phe
        35                  40                  45

Pro Asp Gly Val Gln Asn Trp Pro Ala Leu Ser Ile Val Ile Ile Ile
    50                  55                  60

Ile Met Thr Ile Gly Gly Asn Ile Leu Val Ile Met Ala Val Ser Met
```

65 70 75 80

Glu Lys Lys Leu His Asn Ala Thr Asn Tyr Phe Leu Met Ser Leu Ala
85 90 95

Ile Ala Asp Met Leu Val Gly Leu Leu Val Met Pro Leu Ser Leu Leu
100 105 110

Ala Ile Leu Tyr Asp Tyr Val Trp Pro Leu Pro Arg Tyr Leu Cys Pro
115 120 125

Val Trp Ile Ser Leu Asp Val Leu Phe Ser Thr Ala Ser Ile Met His
130 135 140

Leu Cys Ala Ile Ser Leu Asp Arg Tyr Val Ala Ile Arg Asn Pro Ile
145 150 155 160

Glu His Ser Arg Phe Asn Ser Arg Thr Lys Ala Ile Met Lys Ile Ala
165 170 175

Ile Val Trp Ala Ile Ser Ile Gly Val Ser Val Pro Ile Pro Val Ile
180 185 190

Gly Leu Arg Asp Glu Glu Lys Val Phe Val Asn Asn Thr Thr Cys Val
195 200 205

Leu Asn Asp Pro Asn Phe Val Leu Ile Gly Ser Phe Val Ala Phe Phe
210 215 220

Ile Pro Leu Thr Ile Met Val Ile Thr Tyr Cys Leu Thr Ile Tyr Val
225 230 235 240

Leu Arg Arg Gln Ala Leu Met Leu Leu His Gly His Thr Glu Glu Pro
245 250 255

Pro Gly Leu Ser Leu Asp Phe Leu Lys Cys Cys Lys Arg Asn Thr Ala
260 265 270

Glu Glu Glu Asn Ser Ala Asn Pro Asn Gln Asp Gln Asn Ala Arg Arg
275 280 285

Arg Lys Lys Lys Glu Arg Arg Pro Arg Gly Thr Met Gln Ala Ile Asn
290 295 300

Asn Glu Arg Lys Ala Lys Lys Val Leu Gly Ile Val Phe Phe Val Phe
305 310 315 320

Leu Ile Met Trp Cys Pro Phe Phe Ile Thr Asn Ile Leu Ser Val Leu
325 330 335

Cys Glu Lys Ser Cys Asn Gln Lys Leu Met Glu Lys Leu Leu Asn Val
340 345 350

Phe Val Trp Ile Gly Tyr Val Cys Ser Gly Ile Asn Pro Leu Val Tyr
355 360 365

Thr Leu Phe Asn Lys Ile Tyr Arg Arg Ala Phe Ser Asn Tyr Leu Arg
370 375 380

Cys Asn Tyr Lys Val Glu Lys Lys Pro Pro Val Arg Gln Ile Pro Arg
385 390 395 400

```
Val Ala Ala Thr Ala Leu Ser Gly Arg Glu Leu Asn Val Asn Ile Tyr
                405                 410                 415

Arg His Thr Asn Glu Pro Val Ile Glu Lys Ala Ser Asp Asn Glu Pro
            420                 425                 430

Gly Ile Glu Met Gln Val Glu Asn Leu Glu Leu Pro Val Asn Pro Ser
        435                 440                 445

Ser Val Val Ser Glu Arg Ile Ser Ser Val
    450                 455
```

(31) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1437 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
ATGGATATTC TTTGTGAAGA AAATACTTCT TTGAGCTCAA CTACGAACTC CCTAATGCAA      60
TTAAATGATG ACAACAGGCT CTACAGTAAT GACTTTAACT CCGGAGAAGC TAACACTTCT     120
GATGCATTTA ACTGGACAGT CGACTCTGAA AATCGAACCA ACCTTTCCTG TGAAGGGTGC     180
CTCTCACCGT CGTGTCTCTC CTTACTTCAT CTCCAGGAAA AAAACTGGTC TGCTTTACTG     240
ACAGCCGTAG TGATTATTCT AACTATTGCT GGAAACATAC TCGTCATCAT GGCAGTGTCC     300
CTAGAGAAAA AGCTGCAGAA TGCCACCAAC TATTTCCTGA TGTCACTTGC CATAGCTGAT     360
ATGCTGCTGG GTTTCCTTGT CATGCCCGTG TCCATGTTAA CCATCCTGTA TGGGTACCGG     420
TGGCCTCTGC CGAGCAAGCT TTGTGCAGTC TGGATTTACC TGGACGTGCT CTTCTCCACG     480
GCCTCCATCA TGCACCTCTG CGCCATCTCG CTGGACCGCT ACGTCGCCAT CCAGAATCCC     540
ATCCACCACA GCCGCTTCAA CTCCAGAACT AAGGCATTTC TGAAAATCAT TGCTGTTTGG     600
ACCATATCAG TAGGTATATC CATGCCAATA CCAGTCTTTG GGCTACAGGA CGATTCGAAG     660
GTCTTTAAGG AGGGGAGTTG CTTACTCGCC GATGATAACT TTGTCCTGAT CGGCTCTTTT     720
GTGTCATTTT TCATTCCCTT AACCATCATG GTGATCACCT ACTTTCTAAC TATCAAGGTT     780
CTGCGCCGAC AAGCTTTGAT GTTACTGCAC GGCCACACCG AGGAACCGCC TGGACTAAGT     840
CTGGATTTCC TGAAGTGCTG CAAGAGGAAT ACGGCCGAGG AAGAGAACTC TGCAAACCCT     900
AACCAAGACC AGAACGCACG CCGAAGAAAG AAGAAGGAGA GACGTCCTAG GGGCACCATG     960
CAGGCTATCA ACAATGAAAG AAAAGCTTCG AAGGTACTGG GCATCGTCTT CTTCCTGTTT    1020
GTGGTGATGT GGTGCCCTTT CTTCATCACA AACATCATGG CCGTCATCTG CAAAGAGTCC    1080
```

TGCAATGAGG ATGTCATTGG GGCCCTGCTC AATGTGTTTG TTTGGATCGG TTATCTCTCT 1140

TCAGCAGTCA ACCCACTAGT CTATACTCTG TTCAACAAAA TTTACCGAAG GGCATTCTCC 1200

AACTATTTGC GTTGCAATTA TAAGGTAGAG AAAAAGCCTC CTGTCAGGCA GATTCCAAGA 1260

GTTGCCGCCA CTGCTTTGTC TGGGAGGGAG CTTAATGTTA ACATTTATCG GCATACCAAT 1320

GAACCGGTGA TCGAGAAAGC CAGTGACAAT GAGCCCGGTA TAGAGATGCA AGTTGAGAAT 1380

TTAGAGTTAC CAGTAAATCC CTCCAGTGTG GTTAGCGAAA GGATTAGCAG TGTGTGA 1437

(32) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 478 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Met Asp Ile Leu Cys Glu Glu Asn Thr Ser Leu Ser Ser Thr Thr Asn
1 5 10 15

Ser Leu Met Gln Leu Asn Asp Asp Asn Arg Leu Tyr Ser Asn Asp Phe
20 25 30

Asn Ser Gly Glu Ala Asn Thr Ser Asp Ala Phe Asn Trp Thr Val Asp
35 40 45

Ser Glu Asn Arg Thr Asn Leu Ser Cys Glu Gly Cys Leu Ser Pro Ser
50 55 60

Cys Leu Ser Leu Leu His Leu Gln Glu Lys Asn Trp Ser Ala Leu Leu
65 70 75 80

Thr Ala Val Val Ile Ile Leu Thr Ile Ala Gly Asn Ile Leu Val Ile
85 90 95

Met Ala Val Ser Leu Glu Lys Lys Leu Gln Asn Ala Thr Asn Tyr Phe
100 105 110

Leu Met Ser Leu Ala Ile Ala Asp Met Leu Leu Gly Phe Leu Val Met
115 120 125

Pro Val Ser Met Leu Thr Ile Leu Tyr Gly Tyr Arg Trp Pro Leu Pro
130 135 140

Ser Lys Leu Cys Ala Val Trp Ile Tyr Leu Asp Val Leu Phe Ser Thr
145 150 155 160

Ala Ser Ile Met His Leu Cys Ala Ile Ser Leu Asp Arg Tyr Val Ala
165 170 175

Ile Gln Asn Pro Ile His His Ser Arg Phe Asn Ser Arg Thr Lys Ala
180 185 190

```
Phe Leu Lys Ile Ile Ala Val Trp Thr Ile Ser Val Gly Ile Ser Met
        195                 200                 205

Pro Ile Pro Val Phe Gly Leu Gln Asp Asp Ser Lys Val Phe Lys Glu
    210                 215                 220

Gly Ser Cys Leu Leu Ala Asp Asp Asn Phe Val Leu Ile Gly Ser Phe
225                 230                 235                     240

Val Ser Phe Phe Ile Pro Leu Thr Ile Met Val Ile Thr Tyr Phe Leu
                245                 250                 255

Thr Ile Lys Val Leu Arg Arg Gln Ala Leu Met Leu Leu His Gly His
            260                 265                 270

Thr Glu Glu Pro Pro Gly Leu Ser Leu Asp Phe Leu Lys Cys Cys Lys
        275                 280                 285

Arg Asn Thr Ala Glu Glu Glu Asn Ser Ala Asn Pro Asn Gln Asp Gln
    290                 295                 300

Asn Ala Arg Arg Arg Lys Lys Lys Glu Arg Arg Pro Arg Gly Thr Met
305                 310                 315                     320

Gln Ala Ile Asn Asn Glu Arg Lys Ala Ser Lys Val Leu Gly Ile Val
                325                 330                 335

Phe Phe Leu Phe Val Val Met Trp Cys Pro Phe Phe Ile Thr Asn Ile
            340                 345                 350

Met Ala Val Ile Cys Lys Glu Ser Cys Asn Glu Asp Val Ile Gly Ala
        355                 360                 365

Leu Leu Asn Val Phe Val Trp Ile Gly Tyr Leu Ser Ser Ala Val Asn
    370                 375                 380

Pro Leu Val Tyr Thr Leu Phe Asn Lys Ile Tyr Arg Arg Ala Phe Ser
385                 390                 395                     400

Asn Tyr Leu Arg Cys Asn Tyr Lys Val Glu Lys Lys Pro Pro Val Arg
                405                 410                 415

Gln Ile Pro Arg Val Ala Ala Thr Ala Leu Ser Gly Arg Glu Leu Asn
            420                 425                 430

Val Asn Ile Tyr Arg His Thr Asn Glu Pro Val Ile Glu Lys Ala Ser
        435                 440                 445

Asp Asn Glu Pro Gly Ile Glu Met Gln Val Glu Asn Leu Glu Leu Pro
    450                 455                 460

Val Asn Pro Ser Ser Val Val Ser Glu Arg Ile Ser Ser Val
465                 470                 475
```

(33) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1437 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

ATGGATATTC TTTGTGAAGA AAATACTTCT TTGAGCTCAA CTACGAACTC CCTAATGCAA 60
TTAAATGATG ACAACAGGCT CTACAGTAAT GACTTTAACT CCGGAGAAGC TAACACTTCT 120
GATGCATTTA ACTGGACAGT CGACTCTGAA AATCGAACCA ACCTTTCCTG TGAAGGGTGC 180
CTCTCACCGT CGTGTCTCTC CTTACTTCAT CTCCAGGAAA AAAACTGGTC TGCTTTACTG 240
ACAGCCGTAG TGATTATTCT AACTATTGCT GGAAACATAC TCGTCATCAT GGCAGTGTCC 300
CTAGAGAAAA AGCTGCAGAA TGCCACCAAC TATTTCCTGA TGTCACTTGC CATAGCTGAT 360
ATGCTGCTGG GTTTCCTTGT CATGCCCGTG TCCATGTTAA CCATCCTGTA TGGGTACCGG 420
TGGCCTCTGC CGAGCAAGCT TTGTGCAGTC TGGATTTACC TGGACGTGCT CTTCTCCACG 480
GCCTCCATCA TGCACCTCTG CGCCATCTCG CTGGACCGCT ACGTCGCCAT CCAGAATCCC 540
ATCCACCACA GCCGCTTCAA CTCCAGAACT AAGGCATTTC TGAAAATCAT TGCTGTTTGG 600
ACCATATCAG TAGGTATATC CATGCCAATA CCAGTCTTTG GGCTACAGGA CGATTCGAAG 660
GTCTTTAAGG AGGGGAGTTG CTTACTCGCC GATGATAACT TTGTCCTGAT CGGCTCTTTT 720
GTGTCATTTT TCATTCCCCT GACGATTATG GTGATTACGT ATTGCCTGAC CATCTACGTT 780
CTGCGCCGAC AAGCTTTGAT GTTACTGCAC GGCCACACCG AGGAACCGCC TGGACTAAGT 840
CTGGATTTCC TGAAGTGCTG CAAGAGGAAT ACGGCCGAGG AAGAGAACTC TGCAAACCCT 900
AACCAAGACC AGAACGCACG CCGAAGAAAG AAGAAGGAGA GACGTCCTAG GGGCACCATG 960
CAGGCTATCA ACAATGAAAG AAAAGCTAAG AAAGTCCTTG GGATTGTTTT CTTTGTGTTT 1020
CTGATCATGT GGTGCCCTTT CTTCATCACA AACATCATGG CCGTCATCTG CAAAGAGTCC 1080
TGCAATGAGG ATGTCATTGG GGCCCTGCTC AATGTGTTTG TTTGGATCGG TTATCTCTCT 1140
TCAGCAGTCA ACCCACTAGT CTATACTCTG TTCAACAAAA TTTACCGAAG GGCATTCTCC 1200
AACTATTTGC GTTGCAATTA TAAGGTAGAG AAAAAGCCTC CTGTCAGGCA GATTCCAAGA 1260
GTTGCCGCCA CTGCTTTGTC TGGGAGGGAG CTTAATGTTA ACATTTATCG GCATACCAAT 1320
GAACCGGTGA TCGAGAAAGC CAGTGACAAT GAGCCCGGTA TAGAGATGCA AGTTGAGAAT 1380
TTAGAGTTAC CAGTAAATCC CTCCAGTGTG GTTAGCGAAA GGATTAGCAG TGTGTGA 1437

(34) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 478 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Met Asp Ile Leu Cys Glu Glu Asn Thr Ser Leu Ser Ser Thr Thr Asn
1   5   10   15

Ser Leu Met Gln Leu Asn Asp Asp Asn Arg Leu Tyr Ser Asn Asp Phe
            20   25   30

Asn Ser Gly Glu Ala Asn Thr Ser Asp Ala Phe Asn Trp Thr Val Asp
        35   40   45

Ser Glu Asn Arg Thr Asn Leu Ser Cys Glu Gly Cys Leu Ser Pro Ser
    50   55   60

Cys Leu Ser Leu Leu His Leu Gln Glu Lys Asn Trp Ser Ala Leu Leu
65   70   75   80

Thr Ala Val Val Ile Ile Leu Thr Ile Ala Gly Asn Ile Leu Val Ile
                85   90   95

Met Ala Val Ser Leu Glu Lys Lys Leu Gln Asn Ala Thr Asn Tyr Phe
            100   105   110

Leu Met Ser Leu Ala Ile Ala Asp Met Leu Leu Gly Phe Leu Val Met
        115   120   125

Pro Val Ser Met Leu Thr Ile Leu Tyr Gly Tyr Arg Trp Pro Leu Pro
    130   135   140

Ser Lys Leu Cys Ala Val Trp Ile Tyr Leu Asp Val Leu Phe Ser Thr
145   150   155   160

Ala Ser Ile Met His Leu Cys Ala Ile Ser Leu Asp Arg Tyr Val Ala
                165   170   175

Ile Gln Asn Pro Ile His His Ser Arg Phe Asn Ser Arg Thr Lys Ala
            180   185   190

Phe Leu Lys Ile Ile Ala Val Trp Thr Ile Ser Val Gly Ile Ser Met
        195   200   205

Pro Ile Pro Val Phe Gly Leu Gln Asp Asp Ser Lys Val Phe Lys Glu
    210   215   220

Gly Ser Cys Leu Leu Ala Asp Asp Asn Phe Val Leu Ile Gly Ser Phe
225   230   235   240

Val Ser Phe Phe Ile Pro Leu Thr Ile Met Val Ile Thr Tyr Cys Leu
                245   250   255

Thr Ile Tyr Val Leu Arg Arg Gln Ala Leu Met Leu Leu His Gly His
            260   265   270

Thr Glu Glu Pro Pro Gly Leu Ser Leu Asp Phe Leu Lys Cys Cys Lys
        275   280   285

Arg Asn Thr Ala Glu Glu Glu Asn Ser Ala Asn Pro Asn Gln Asp Gln

290 295 300

Asn Ala Arg Arg Arg Lys Lys Lys Glu Arg Arg Pro Arg Gly Thr Met
305 310 315 320

Gln Ala Ile Asn Asn Glu Arg Lys Ala Lys Lys Val Leu Gly Ile Val
325 330 335

Phe Phe Val Phe Leu Ile Met Trp Cys Pro Phe Phe Ile Thr Asn Ile
340 345 350

Met Ala Val Ile Cys Lys Glu Ser Cys Asn Glu Asp Val Ile Gly Ala
355 360 365

Leu Leu Asn Val Phe Val Trp Ile Gly Tyr Leu Ser Ser Ala Val Asn
370 375 380

Pro Leu Val Tyr Thr Leu Phe Asn Lys Ile Tyr Arg Arg Ala Phe Ser
385 390 395 400

Asn Tyr Leu Arg Cys Asn Tyr Lys Val Glu Lys Lys Pro Pro Val Arg
405 410 415

Gln Ile Pro Arg Val Ala Ala Thr Ala Leu Ser Gly Arg Glu Leu Asn
420 425 430

Val Asn Ile Tyr Arg His Thr Asn Glu Pro Val Ile Glu Lys Ala Ser
435 440 445

Asp Asn Glu Pro Gly Ile Glu Met Gln Val Glu Asn Leu Glu Leu Pro
450 455 460

Val Asn Pro Ser Ser Val Val Ser Glu Arg Ile Ser Ser Val
465 470 475

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08168

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C07H 21/04; C07K 14/705; C12N 15/09, 15/63; C12Q 1/68
US CL : 536/23.5, 24.3; 435/7.1, 69.1, 320.1; 530/350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 536/23.5, 24.3; 435/7.1, 69.1, 320.1; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, MEDLINE, HCAPLUS, BIOSIS, EMBASE, JAPIO, WPIDS
search terms: sertonin receptor, 5ht2, agonist, antagonist, inverse agonist, chemical structure of compound A, B, i, II, III.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HARTMAN, J.L. et al. Functional Reconstitution in Situ of 5-Hydroxytryptamine 2c (5HT2c) Receptors with Alpha-q and Inverse Agonism of 5HT2c Receptor Antagonists. J. Biol. Chem. 13 September 1996, Vol. 271, No. 37, pages 22591-22597, see entire document.	7
A,P	WO 98/24785 A1 (FUGI-SAWA PHARMACEUTICAL CO., LTD.) 11 June 1998, see entire document.	7, 18, 19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
26 JULY 1999

Date of mailing of the international search report
28 AUG 1999

Name and mailing address of the ISA/US
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D. Lawrence for
NIRMAL S. BASI
Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08168

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-6, 8-10 and 15-17
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 1-6 and 8-10 require a sequence search of the amino acid sequence of the protein or nucleotide search of the polynucleotide. The sequences in computer readable form were defective. Claims 15-17 are not present in the application.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
7, 18 and 19

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)7, drawn to a method for identifying whether a candidate compound is an inverse agonist to a non-endogenous human 5HT2 serotonin receptor.
Group II, claim(s) 11, drawn to an agonist to a non-endogenous human 5HT2 serotonin receptor.
Group III, claim(s) 12 and 13, drawn to a reagent for screening compounds to determine whether the compounds are inverse agonists to human 5HT2 serotonin receptor.
Group IV, claim(s)14, drawn to a method for modulating by inverse agonism the activity of a human 5HT2a serotonin receptor by contacting the receptor with the compound A.
Group V, claim(s) 18, drawn to a method for modulating by inverse agonism the activity of a human 5HT2a serotonin receptor by contacting the receptor with the compound B.
Group VI, claim(s) 19, drawn to a method for modulating by inverse agonism the activity of a human 5HT2a serotonin receptor by contacting the receptor with the compound I.
Group VII, claim(s)20, drawn to a method for modulating by inverse agonism the activity of a human 5HT2a serotonin receptor by contacting the receptor with the compound II.
Group VIII, claim(s) 20, drawn to a method for modulating by inverse agonism the activity of a human 5HT2a serotonin receptor by contacting the receptor with the compound III.
Group IX, claim(s) 21 and 22, drawn to compound C and use for manufacture of a medicament.
Claims 15-17 are not grouped because they were not present in the application.
The inventions listed as Groups I-IX do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The special technical feature of Group I is the method for identifying whether a candidate compound is an inverse agonist to a non-endogenous human 5HT2 serotonin receptor. Pursuant 37 CFR 1.474 (d), the claim is considered by the ISA/US to constitute the main invention, and none of the related Groups I-IX correspond to the main invention. Prior art teaches the special technical feature of Group I. Hartman et al (J. Biol. Chem., 1996, Vol. 271, No.3, pages 22591-22597) disclose a method for identifying whether a candidate compound is an inverse agonist to a non-endogenous human 5HT2 serotonin receptor. The method of Group I does not provide an advance over the prior art. The methods of Groups IV-VIII do not share a special technical feature in any pairing because the special technical feature is the inverse agonist, and each method is practiced by a different inverse agonist. The products of Groups II, III and IX do not share the technical feature in any pairing because the compounds are structurally different and capable of separate use and manufacture.